



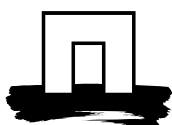
## Report 34

# Feeding strategies to reduce methane loss in cattle



February 2007





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### **Abstract**

This report presents an overview of the enteric methane production in cattle. The possibilities are discussed to influence methane production by feeding measures and the use of feed additives, and by management measures. The possibilities are discussed against the background of Dutch cattle husbandry and points for attention and priorities for future research have been formulated.

### **Keywords**

methane, cattle, feeding strategies, models

### **Referaat**

Dit rapport geeft een overzicht van de methaanvorming in het maagdarmkanaal van rundvee. De mogelijkheden worden besproken om de methaanvorming te beïnvloeden door middel van voedingsmaatregelen en het gebruik van voeradditieven, en door middel van managementmaatregelen. Deze mogelijkheden zijn besproken in de context van de Nederlandse rundveehouderij en op basis hiervan zijn aandachtspunten en prioriteiten voor toekomstig onderzoek geformuleerd.

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### **Trefwoorden:**

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## Report 34

# Feeding strategies to reduce methane loss in cattle

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## Samenvatting

Het rapport geeft eerst een overzicht van de principes en achtergronden van methaan ( $\text{CH}_4$ ) productie door melkkoeien. Aspecten die de revue passeren zijn de functie van en microbiële activiteit in de pens en de dikke darm. De microbiële activiteit mondt uit in het omzetten van door de koe opgenomen voer in microbiële massa, vluchtige vetzuren (VFA), fermentatiewarmte en de fermentatiegassen koolstofdioxide ( $\text{CO}_2$ ) en methaan ( $\text{CH}_4$ ). Het belang wordt benadrukt van de vorming van  $\text{CH}_4$  als gevolg van het moeten handhaven van de waterstof ( $\text{H}_2$ ) balans in de anaërobe pens, welke op haar beurt wordt beïnvloed door de opbrengst en soort van VFA. De vorming van azijnzuur (HAc) en boterzuur (HBu) gaat gepaard met een overschot aan  $\text{H}_2$ , de vorming van propionzuur (HPr) gaat daarentegen gepaard met de opname van  $\text{H}_2$ . Het hoofdstuk wordt afgesloten met een overzicht van de belangrijkste factoren die van invloed zijn op de  $\text{CH}_4$  vorming. Dit zijn voeropname niveau van de koe, intrinsieke afbraak karakteristieken van voer fracties, type substraat (structurele koolhydraten of ruwvezel en suikers vs. de niet-structurele koolhydraten zetmeel) en het effect van de zuurtegraad (pH) in de pens.

Het volgende onderdeel van het rapport bespreekt een aantal modellen welke de vorming van  $\text{CH}_4$  beschrijven (simuleren) en voorspellen. Allereerst wordt uitgelegd waarom modellen nodig zijn en worden een aantal algemene opmerkingen gemaakt over overwegingen die een rol spelen bij het kiezen van het best passende model. Dit wordt gevolgd door een bespreking van een 4-tal types modellen, t.w. statisch empirische modellen, dynamisch empirische modellen, dynamisch mechanistische modellen en ten slotte bedrijfs (farm budget) modellen. Er wordt de conclusie getrokken dat voor het bereiken van een enigszins nauwkeurige schatting van de productie van  $\text{CH}_4$  op dierniveau, dynamische mechanistische modellen het meest geëigend zijn. Dit type modellen zou vervolgens ook het startpunt dienen te zijn voor bedrijfsmodellen.

Het rapport geeft vervolgens een overzicht van de stand van zaken m.b.t.  $\text{CH}_4$  productie door melkvee in Nederland in 2006 via een korte beschrijving van recente (2000-2005) en te verwachten toekomstige (2006-2010) ontwikkelingen van de rundveehouderij in Nederland. De huidige  $\text{CH}_4$  emissie door de gemiddelde Nederlandse melkkoe lijkt de default waarde die het IPCC (1996) hanteert dicht te naderen, en komt overeen met ongeveer 15 g  $\text{CH}_4$  per kg geproduceerde melk. Startpunt hierbij zijn de conclusies en aanbevelingen zoals geformuleerd door Veen (2000), aangevuld met die van enkele daarna nog verschenen rapporten. Verwacht wordt dat de melkproductie per koe zal blijven stijgen met als gevolg gelijktijdig een afname van de  $\text{CH}_4$  verliezen per kg melk. Voor het jaar 2010 wordt dan ten opzichte van 2003 een daling verwacht van ruim 5%. Verder wordt een afname verwacht van het aantal dieren nodig voor het vervangen van de veestapel, waardoor ook de  $\text{CH}_4$  verliezen per kg melk licht, met 2%, zullen dalen. Echter, het jaarrond opstallen van melkvee zal vermoedelijk verder toenemen. Ten slotte wordt beargumenteerd dat, als gevolg van een lagere N bemesting, de kwaliteit van de ruwvoerders die gebruikt worden voor de voeding van melkvee, zullen tenderen naar een afname en niet naar een toename. De meeste van deze ontwikkelingen zullen de  $\text{CH}_4$  verliezen eerder doen toe- dan afnemen. De effecten die verwacht mogen worden zijn echter onzeker vanwege variatie in de rantsoen karakteristieken, ruwvoer kwaliteit, voeropnameniveau, huisvestingssystemen en de extra bijdrage van indirecte  $\text{CH}_4$  emissie uit de mestopslag.

Het rapport gaat door met een uitgebreide analyse van mogelijke voedingsstrategieën die de  $\text{CH}_4$  productie door melkvee kunnen verminderen. Een zeer effectieve maatregel is het verhogen van het aandeel snijmais in ruwvoer ten koste van grassilage. Toepasbaarheid van deze maatregel wordt echter belemmerd door de huidige mestwetgeving en ook bestaat het risico op afwenteling naar overige broeikasgassen. De invloed van een verschuiving van begrazing naar stalvoeding van grassilage is voornamelijk nog onduidelijk en dient verder onderzocht te worden. Ingrepen op het gebied van ruwvoederproductie (vers gras, grassilage, gehele planten graansilage en vlinderbloemige planten) worden geacht slechts een marginaal effect te hebben op de  $\text{CH}_4$  uitstoot. Bij maaisilage lijkt er in potentie iets meer winst geboekt te kunnen worden door variëteiten te gebruiken die hetzij een hoger gehalte aan (pensbestendig) zetmeel hebben, hetzij een hoger gehalte aan olie. Vermindering van  $\text{CH}_4$  verliezen zullen echter beperkt blijven tot minder dan 5%. Voor wat betreft de krachtvoerders verwachten de auteurs van het rapport dat de hoeveelheid per koe verstrekt krachtvoer nog wel enigszins zal toenemen. Ten aanzien van de krachtvoersamenstelling kan het uitkiend selecteren van grondstoffen die rijk zijn aan specifieke types koolhydraten, zoals meer pensbestendig zetmeel en minder oplosbare suikers, leiden tot een betekenisvolle vermindering van de  $\text{CH}_4$  emissie. Daarnaast kan het in het krachtvoer opnemen van specifieke vetten en oliën een bijdrage leveren aan de reductie in de uitstoot van  $\text{CH}_4$ . Geschat wordt dat veranderingen in de krachtvoersamenstelling de  $\text{CH}_4$  verliezen voornamelijk met niet meer dan 10% kunnen verminderen. Om de effecten van voeder additieven (organische zuren, etherische oliën) en secundaire metabolieten in planten (gecondenseerde tannines, saponines), vast te stellen is er nog veel onderzoek nodig voordat dit soort toevoegingen in de praktijk bruikbaar zijn. Veel van het onderzoek op dit gebied is gebaseerd op kortdurende *in vivo* en *in vitro* studies met gerapporteerde afnames van  $\text{CH}_4$  met meer dan 30%, maar positieve effecten *in vivo* worden nog zelden waargenomen. Vooral de afwezigheid van duurzaamheid van de effecten, als gevolg van het zich aan de nieuwe omstandigheden aanpassen van de pensfermentatie onder praktische *in vivo* omstandigheden

lijkt de vooruitzichten op dit gebied te beperken. Mogelijk dat uitgekende veranderingsstrategieën in de voeding dit probleem kunnen helpen oplossen.

Het rapport besteedt ook aandacht aan mogelijke bijkomende effecten van de pogingen om  $\text{CH}_4$  verliezen te beperken. Rantsoenveranderingen kunnen zowel een gunstige als een negatieve uitwerking hebben op de hoeveelheid en samenstelling van de melk. De gehalten aan eiwit en lactose in de melk zullen nauwelijks beïnvloed worden, maar de samenstelling van het melkvet lijkt te kunnen worden beïnvloed in een richting die gunstig geacht wordt voor de gezondheid van de mens. Sommige van de voerstrategieën die gericht zijn op het verminderen van de  $\text{CH}_4$  uitstoot (vooral strategieën die het aanbod aan glucogene nutrienten verhogen, zoals meer zetmeel of meer bestendig zetmeel) kunnen ook gunstig zijn voor de diergezondheid. Dit is met name het geval in het begin van de lactatie als ze de omvang van de Negatieve Energie Balans (NEB) helpen beperken. Het laatste deel van dit onderdeel van het rapport handelt over het mogelijke effect dat voerstrategieën, die uitstoot van  $\text{CH}_4$  beogen te verminderen, kunnen hebben op de uitstoot van andere broeikasgassen. Een succesvolle strategie die de  $\text{CH}_4$  uitstoot vermindert gaat niet zelden gepaard met een verhoging van de uitstoot van  $\text{CO}_2$  en/of  $\text{N}_2\text{O}$ . Benadrukt wordt dat het zich eenzijdig richten op het terugdringen van de uitstoot van een enkel broeikasgas tot gevolg kan hebben dat het probleem wordt verplaatst van het ene broeikasgas naar het andere. In dit kader wordt ook aanbevolen een nadere studie te doen naar de met de productie en het transport van voeders naar melkveebedrijven gepaard gaande verliezen aan  $\text{CO}_2$  als gevolg van een hogere input van fossiele energie. Bovendien dient onderzocht te worden in welke mate het verminderen van de  $\text{CH}_4$  uitstoot gepaard gaat met een toename of juist afname van de  $\text{NH}_3$  emissie.

Het rapport eindigt met een discussie over de mogelijkheden om de in het rapport besproken maatregelen voor een verminderde  $\text{CH}_4$ -uitstoot in de praktijk toe te passen. Ze worden onderscheiden in aanbevelingen voor koeien in het begin van de lactatie, koeien halverwege de lactatie, koeien op het einde van de lactatie en droogstaande koeien en jonge dieren die worden aangehouden ter vervanging van de veestapel. Aanbevolen wordt om bij koeien in het begin van de lactatie extra pensbestendig zetmeel in het rantsoen op te nemen. Koeien die halverwege de lactatie zijn zouden wat extra vet in hun rantsoen moeten hebben en koeien aan het eind van de lactatie en in de droogstand zouden gebaat zijn bij ruwvoerders met een hoger dan normaal gehalte aan gecondenseerde tannines in hun rantsoen. Ook wordt aanbevolen om te onderzoeken of het in de praktijk loont om jonge dieren intensiever te voeren, zodat ze sneller groeien en hun ideale afkalfleeftijd 1 tot 2 maanden eerder bereiken, en daarmee minder bijdragen aan de  $\text{CH}_4$ -emissie.

Het rapport wordt afgesloten met het formuleren van een aantal algemene conclusies en een tabel waarin de mogelijke voedingstechnische interventies en hun effecten op de uitstoot van  $\text{CH}_4$  worden samengevat.

Resumerend wordt benadrukt dat strategieën die tot doel hebben de uitstoot van broeikasgassen te beperken, geïntegreerd en op bedrijfsniveau moeten worden uitgevoerd. Er wordt voor gewaarschuwd dat interventies die de uitstoot van het ene broeikasgas verminderen niet zelden tot gevolg hebben dat die van een ander broeikasgas toenemen. Omdat de huidige in gebruik zijnde veelal empirische modellen niet goed de effecten van interventies ter vermindering van de uitstoot van broeikasgassen kunnen schatten, wordt aanbevolen te werken met dynamische mechanistische modellen.

Er wordt ook voor gewaarschuwd dat veel studies zijn gebaseerd op kortdurende *in vitro* experimenten. Voordat de resultaten toepasbaar zijn dienen ze te worden getoetst in langlopende *in vivo* experimenten. Betoogd wordt dat  $\text{CH}_4$  verliezen door melkvee in Nederland waarschijnlijk al tot de laagste ter wereld behoren en dat ze de komende jaren als gevolg van de autonoom lijkende stijging van de melkproductie per kg melk nog verder zullen afnemen. Door te sleutelen aan de voersamenstelling in de richting van meer pensbestendig zetmeel en het in het krachtvoer opnemen van specifieke onverzadigde vetzuren kan er nog enige extra reductie in  $\text{CH}_4$  uitstoot worden bereikt onder de huidige productieomstandigheden. Wel wordt geconcludeerd dat er onvoldoende kennis is op het gebied van het gedrag van afbraak en passage in het door het maagdarmkanaal en daaruit voortvloeiend de plaats van vertering en het profiel van de gevormde VFA. Op dit terrein is meer onderzoek gewenst, met name voor wat betreft zetmeel in maaisilage.

Het ontwikkelen van ruwvoerders met verhoogde gehalten aan onverzadigde oliën of gecondenseerde tannines lijkt ook enig perspectief te bieden, maar ook hier is nog veel onderzoek nodig.

Tot slot wordt in volgorde van prioriteit, een opsomming gegeven van aanbevolen velden en/of onderwerpen voor nader onderzoek.

1. Verteringsgedrag van zetmelen (snijmaïs, krachtvoerders) in het maagdarmkanaal van melkvee
2. Effectiviteit, interacties en additiviteit van verschillende CH<sub>4</sub> reductiemethoden.
3. Effectiviteit van het afwisselen van verschillende CH<sub>4</sub> reductiemethoden (vetten, additieven) gedurende de lactatie (switch-over strategie).
4. Vergelijking van verteerbaarheidskarakteristieken tussen en binnen ruwvoerders (gras, gras silage, snijmaïs silage, GPS, etc.)
5. Monitoren van pensfermentatie, inclusief vetzuursamenstelling (m.n. OBCFA) van melk, in combinatie met en als onderdeel van 1, 2, 3 en 4
6. CH<sub>4</sub> verliezen uit mest en de pens
7. Scenarios van uitruil en afwenteling van broeikasgas emissies
8. Tannines in ruwvoer
9. Jongvee opfokstrategieën

## Summary

The report starts with an overview of the principles and background of enteric methane ( $\text{CH}_4$ ) production in dairy cows. Aspects that are discussed are function and microbial activity in the reticulorumen and the hindgut. Microbial activity results in the conversion of feeds ingested by the cow in microbial mass, volatile fatty acids (VFA) fermentation heat and the fermentation gases carbon dioxide ( $\text{CO}_2$ ) and methane ( $\text{CH}_4$ ). The importance of  $\text{CH}_4$  formation for maintaining the hydrogen ( $\text{H}_2$ ) balance in the anaerobic rumen, as influenced by the yield of volatile fatty acids (VFA), is stressed. The formation of acetic acid (HAc) and butyric acid (HBu) results in a surplus of  $\text{H}_2$ , the formation of propionic acid (HPr) on the other hand is associated with an uptake of  $\text{H}_2$ . The chapter is completed with an account of the factors influencing  $\text{CH}_4$  yield such as level of feed intake by the cow, intrinsic degradation characteristics of feed fractions, type of substrates fermented (structural carbohydrates or fibre and sugars vs the non-structural carbohydrates in starch), and the effect of acidity (pH) in the rumen.

The next section deals with discussing a variety of models simulating and predicting  $\text{CH}_4$  yield. The section starts with explaining why models are needed and with some general considerations in choosing the most appropriate model. This is followed by a discussion of four types of models, static empirical models, dynamic empirical models, dynamic mechanistic models and finally farm budget models. It is concluded that for an accurate prediction of  $\text{CH}_4$  production at animal level and for mitigation strategies to reduce methane emission, dynamic mechanistic models are the best choice. Such models should subsequently be the starting point for farm budget models.

This is followed by giving an account of the state of the art in The Netherlands in 2006 through a brief description of recent (2000-2005) and future (2006-2010) developments with regard to dairy husbandry in The Netherlands. Starting point were the conclusions and recommendations of Veen (2000), complemented with some later reports. Current  $\text{CH}_4$  emission by the average Dutch dairy cow appears close to the default value of 6% of gross energy intake adopted by the IPCC (1996), corresponding to about 15 g of  $\text{CH}_4$  per kg of milk produced. It is expected that milk yield per cow will continue to rise with a concomitant reduction of  $\text{CH}_4$  losses per kg of milk. This could lead to a reduction of over 5% in 2010 as compared to 2003. It is also expected that the number of replacement animals will continue to fall with a concomitant reduction in  $\text{CH}_4$  losses of 2%. However, it is feared that zero grazing will increase, and finally it is argued that, because of lower N fertilisation, the quality of forages for dairy cows will show a tendency to decline rather than to improve. These latter developments will probably increase rather than decrease  $\text{CH}_4$  losses.

The report continues with an extensive account on mitigation strategies for  $\text{CH}_4$  losses in dairy cows, to be realised through nutritional interventions. A highly effective measure is an increased proportion of maize silage at the expense of grass silage. Feasibility of this measure is limited, however, because of current legislation, and effects may be offset by an increase in other green house gases (GHG). The impact of a shift between from grazing towards stall-feeding of grass silage requires further investigation. Effects of interventions at the level of forage production (fresh grass, grass silage, whole cereal plant silage and forage legumes) on  $\text{CH}_4$  losses are considered marginal. For maize silage some potential is seen for varieties with an increased content of rumen resistant starch or a high oil content. Reductions of  $\text{CH}_4$  losses will be limited to less than 5%. When dealing with concentrates, the report assumes a further increase in the amount of concentrate consumption per animal. With regard to the ingredient composition of concentrates, selecting carefully defined carbohydrate fractions, such as more starch of a higher rumen resistance and less soluble sugars could significantly contribute to a reduction in  $\text{CH}_4$  emission. In addition, the inclusion of specific fats and oils could also contribute to this objective. Potential reductions in  $\text{CH}_4$  losses are estimated at 10% at maximum. To establish the effects of the inclusion of feed additives (organic acids, essential oils) and plant secondary metabolites (condensed tannins, saponins), still a substantial amount of research is needed before they can be used for mitigation purposes in practice. Much of the research in this area is based on short term *in vivo* or *in vitro* approaches with reported reductions in  $\text{CH}_4$  loss of over 30%, but positive effects *in vivo* have as yet rarely been established. Notably, a lack of persistency of effects because of adaptation of the rumen fermentation under practical *in vivo* conditions seems to limit prospects in this area. Perhaps carefully designed alternating feeding strategies could circumvent the problem of adaptation.

An account is given of possible side effects of attempts to reduce  $\text{CH}_4$ . Dietary manipulation may have favourable as well as adverse effects on the quantity and quality of milk. The concentrations of protein and lactose will hardly be influenced, but prospects are seen for altering milk fat composition in a direction which is desired for human health. Certain nutritional strategies (in particular strategies that increase the glucogenic nutrient supply, such as more starch or more resistant starch), aiming at a reduction of  $\text{CH}_4$  losses could also stimulate animal health, notably in early lactation by alleviating the Negative Energy Balance (NEB).

The final part of this section deals with the effects mitigation strategies for  $\text{CH}_4$  losses may have on the loss of other green house gases, notably carbon dioxide ( $\text{CO}_2$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ). A successful reduction of  $\text{CH}_4$

losses from cows may be at the expense of an increased loss of CO<sub>2</sub> and N<sub>2</sub>O elsewhere. It is emphasized that targeting at the mitigation of only one GHG may result in a shift in the loss from one GHG to the other. In the scope of this shift it is recommended to make an updated inventory of the input of fossil energy, resulting in an excretion of CO<sub>2</sub>, required for the production and transport of feeds imported on dairy farms. It remains to be investigated to what extent CH<sub>4</sub> mitigation options increase or reduce NH<sub>3</sub> emissions.

Although current dynamic mechanistic models are already capable of representing the effects exerted by feed additives, these models still require further development before they can be applied to predict the effect of additives on rumen fermentation, ruminant performance and CH<sub>4</sub> loss.

There is also scope for the development of a non-invasive tool for monitoring CH<sub>4</sub> losses. A close relationship seems to exist between rumen fermentation pattern and the excretion of odd- and branched-chain fatty acids (OBCFA) in milk. This relationship between OBCFA and CH<sub>4</sub> loss should first be verified, however, before applying this instrument in practice.

At the end of the report, the practical implementation of nutritional interventions to reduce CH<sub>4</sub> losses are discussed. They are separated in recommendations for early lactation cows, mid lactation cows, late lactation and dry cows, and young stock. It is suggested to include more bypass starch in diets for early lactation cows, some extra fat in diets for mid lactation cows and high tannin forage legumes in diets for late lactation and dry cows. It is also suggested to further investigate the applicability in practice of feeding young stock more intensive, to accelerate their growth and have them ready for the first calving 1 to 2 months earlier than normal, leading to less CH<sub>4</sub> from young stock, provided that the accelerated pre-pubertal growth does not negatively impact on milk production in first lactation.

The report is concluded with general conclusions and a table showing an overview of possible interventions and their effect on CH<sub>4</sub>-emission.

In summary, it is emphasized that mitigation strategies for GHG at the animal level also need to be evaluated at the farm level. A warning is issued that interventions that aim to reduce the loss of one GHG may accelerate the loss of another GHG. Because most of the models presently in use are of an empirical nature, it is recommended to use dynamic mechanistic models for the prediction GHG losses and to evaluate mitigation strategies.

It is noted that many recent studies are based on short term *in vitro* experiments. Before the results of such experiments are applicable in practice, they have to be thoroughly evaluated in long term *in vivo* experiments. It is argued that, when expressed per kg of milk, CH<sub>4</sub> losses from dairy cows in the Netherlands are probably among the lowest in the world and that because of an apparently autonomous increase of the milk production per animal they will diminish further. By manipulating the diet composition towards more and more rumen resistant starch and the inclusion of specific unsaturated fatty acids in concentrates an extra reduction of CH<sub>4</sub> losses appears possible under current production conditions. A warning is given that present knowledge on the degradative and passage behaviour of starch in the digestive tract of dairy cows and the resulting VFA profile, is insufficient to realise the full benefit of this potential. More research is needed in this area, notable with regard to starch in maize silage.

The development of forages with elevated levels of unsaturated oils or condensed tannins seems to have some potential, but this still requires further a lot of research.

Finally, a list is included of the important issues that need to be addressed in future research on methane emission by ruminants, and some recommendations are given.

1. Digestive behaviour of starches in the gastrointestinal tract of dairy cows
2. Effectivity, interactions and additivity of CH<sub>4</sub> reducing measures
3. Effectivity of switch-over strategies (fats, additives, etc.) of different CH<sub>4</sub> reducing measures
4. Comparison of digestion characteristics between and among various forages (grass, grass silage, maize silage, GPS, etc.)
5. Monitoring rumen fermentation including the fatty acid profile (notably OBCFA) of milk.
6. Separating enteric CH<sub>4</sub> losses from those of manure in storage
7. Scenario's of trade-offs of GHG emissions
8. Tannins in forages
9. Raising strategies of young stock



# Inhoudsopgave

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## 1 Introduction

The emission of greenhouse gases (GHG), notably of methane ( $\text{CH}_4$ ), by domestic animals and possible ways of abatement have been the subject of many international studies in recent years (Johnson & Johnson, 1995; Moss et al., 2000; Boadi et al., 2004; Kebreab et al. 2006a). From all emission sources of  $\text{CH}_4$ , agriculture is by far the most important source in The Netherlands. Enteric fermentation in ruminants accounts for 75% of the emission and manure management for 25% (Klein Goldewijk et al., 2005). The maximum range of  $\text{CH}_4$  losses from dairy cows appears to be 4.5 to 8.5% of the Gross Energy Intake (GEI). The observations larger than 7% are probably an artefact due to a restricted intake of high quality diets of animals kept in laboratory chambers under experimental conditions and unrealistic for practical conditions (Johnson et al., 1997). For all categories of ruminants the range may be larger. In particular for feedlot cattle fed high grain rations it may be as low as 2 to 3%.

Several techniques to measure  $\text{CH}_4$  losses from farm animals exist. They were recently reviewed by Kebreab et al. (2006b). Most widely used among them are respiration calorimetric chambers and sulphur hexafluoride ( $\text{SF}_6$ ) as a tracer gas. Also, there are several ways to express  $\text{CH}_4$  losses. The most widely used way is to express it as % of GEI. Expressing it as litre per kg of dry matter intake ( $\text{L CH}_4/\text{kg DMI}$ ) is also quite common (Giger-Reverdin et al., 2003). A less popular, but for dairy cows interesting way to express  $\text{CH}_4$  losses is as gram (or litre) per kg desired product, hence  $\text{g CH}_4/\text{kg}$  of milk.

Some years ago a series of research projects on the subject of  $\text{CH}_4$  losses from ruminants in The Netherlands were started, coordinated by the Rob-Agro research programme committee ([www.robklimaat.nl](http://www.robklimaat.nl)). Veen (2000) reviewed the role that animal nutrition could play to alleviate the loss of  $\text{CH}_4$  from ruminant animals, notably from dairy cows. Several Rob-Agro studies have been performed since and evaluated the effect of nutrition and feed additives on  $\text{CH}_4$  emission (Smink et al., 2004; Van Zijderveld & Van Straalen, 2004; Van Laar & Van Straalen, 2004). Simultaneously, a research project, funded by the Dutch Commodity Board of Feedstuffs and the ministry of Agriculture, Nature and Food Quality (LNV), started at the end of 2002 aiming at the quantification of  $\text{CH}_4$  emission by dairy cows by applying an integrative modelling approach. The model developed was recently used to deliver estimates for the national emission of  $\text{CH}_4$  by cows (Smink et al., 2005). These estimates were used in the Dutch national inventory of emission of greenhouse gases from agriculture.

## 2 Principles and background of enteric CH<sub>4</sub> production

Methane originates from anaerobic microbial fermentation processes in the gastrointestinal tract of ruminant animals. This fermentation occurs particularly in the reticulorumen, rumen in short. In an adult cow, the rumen occupies a volume of over 100 litres of which usually 85 to 90% is fluid. The high moisture content and a temperature that is kept rather constant at around 37° C makes this an eminently suited environment for microbes to survive and grow, provided the microbes are regularly supplied with a suitable substrate. Substrates needed by the microbes are provided through the ingestion of feed by the host animal. The feed ingested by a ruminant is attacked by the microbes and degraded in a wide range of end products.

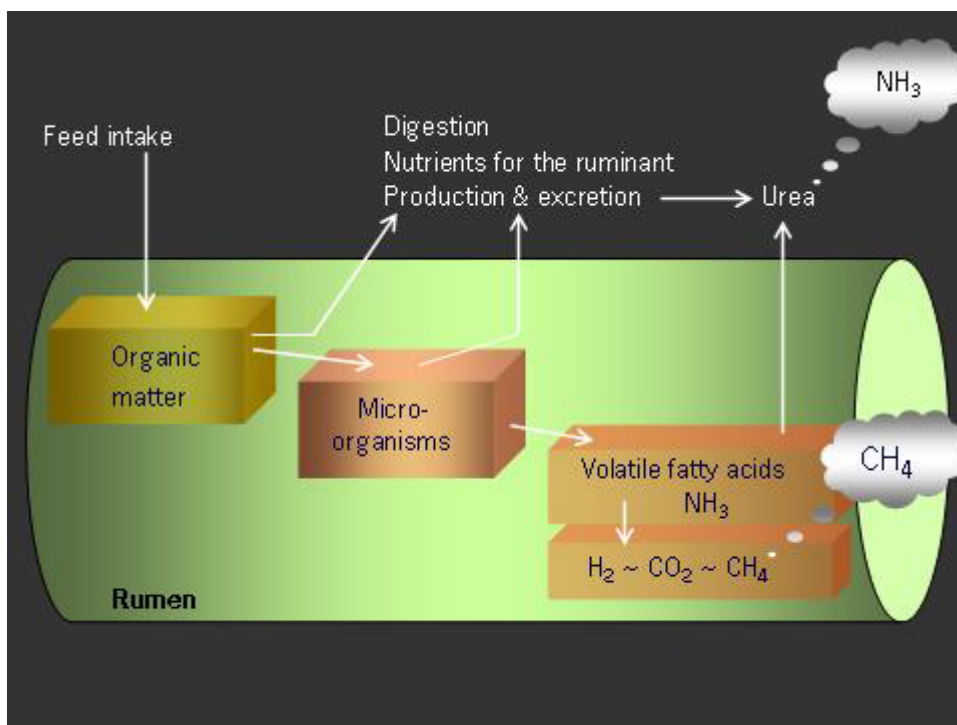
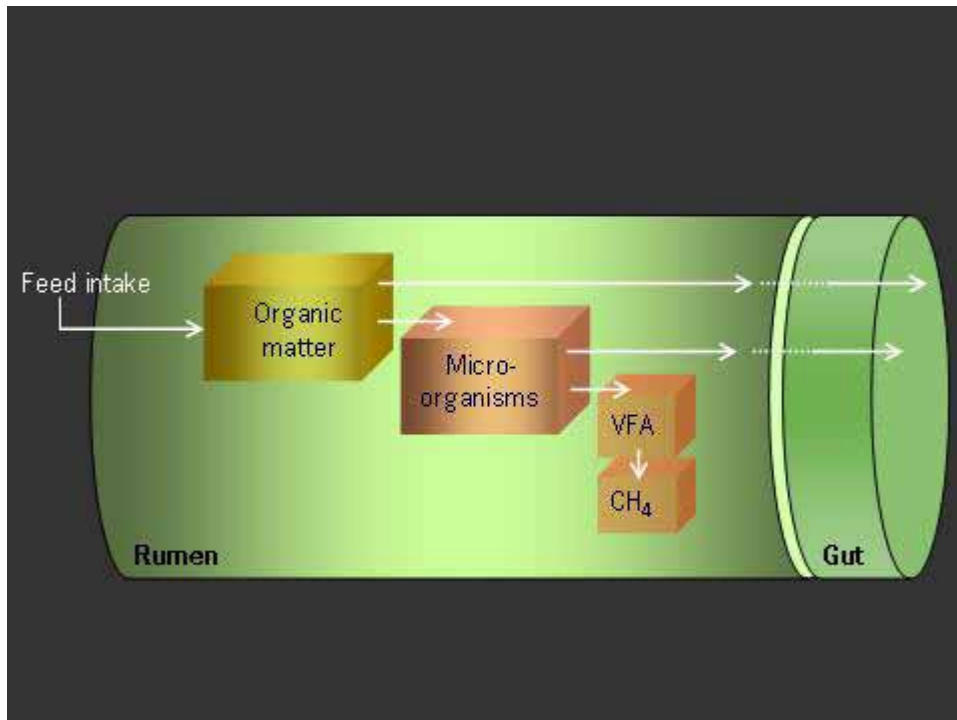
The anaerobic condition in the rumen and hindgut limit the oxidation of organic substrate into carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O), but an internal rearrangement of the carbon (C), hydrogen (H) and oxygen (O) present in the feed, between microbial biomass and the end products, keeps the system going. During this process reducing equivalents (H<sub>2</sub>) are generated. To prevent the accumulation of H<sub>2</sub>, which by itself is poisonous to the microbes, a H<sub>2</sub> sink is needed. Various H<sub>2</sub> sinks are present in the rumen of which the conversion of H<sub>2</sub> and CO<sub>2</sub> into CH<sub>4</sub> is by far the most important. An accumulation of CH<sub>4</sub> is prevented by eructation and respiration, and the CH<sub>4</sub> is emitted to the environment. Other important end products of fermentation are microbial mass and volatile fatty acids (VFA).

### 2.1 Rumen function and microbial growth

The rumen resembles a microbial population cultured in a buffered semi-continuous chemostat with a semi-continuous inflow of substrates from ingested feed as well as inflow of drinking water, and continuous outflow and mixing of contents. There is also a continuous inflow of urea in saliva and by absorption from blood to the rumen. Buffering is maintained by the continuous inflow of saliva and due the process of VFA absorption. Simultaneously, an outflow of unfermented substrates, of synthesized microbial matter and of rumen fluid and solutes occurs. The majority of the VFA (about two thirds) leave the rumen through absorption by the rumen wall to the blood. Mixing of rumen contents takes place by frequent rumen contractions.

The main part, usually some 60 to 65%, of the dry matter entering the rumen is carbohydrates, 15 to 20% is protein, 10 to 15% minerals and less than 10% lipids. Several types of carbohydrates (sugars, starch, pectins, hemicellulose and cellulose) are degraded by the micro-organisms in the rumen. Also degradation of proteins occurs and a significant proportion of their amino acids is either incorporated in microbial protein or degraded further in VFA and ammonia (NH<sub>3</sub>). Lipids and free long-chain fatty acids are hardly fermented, but, to some extent, long chain fatty acids may be incorporated in microbial matter (Harfoot & Hazlewood, 1997). Within the rumen several classes of micro-organisms degrade substrates, synthesize microbial matter, and ferment substrate to VFA, CO<sub>2</sub>, H<sub>2</sub> and NH<sub>3</sub>. The purpose of the degradation is to generate energy for maintenance and energy and precursors for synthetic processes (Figure 1).

**Figure 1** Schematic representation of the effect of nutrition on substrate degradation and the fermentative processes in the rumen



Mostly, a pragmatic and rather global distinction is made between sugar and starch utilizing bacteria, cell wall utilizing bacteria and protozoa, of which the latter predate on bacteria. These microbial classes not only distinguish themselves in the type of substrate they use, but also in the fractional rate of their rumen outflow, together with their preferred types of substrate. Protozoa are capable to retain themselves within the rumen and flow out with a much lower fractional rate than bacteria.

Methanogens co-exist with the substrate degrading micro-organisms and produce  $\text{CH}_4$  from  $\text{CO}_2$  and  $\text{H}_2$ . By far the major part of the  $\text{H}_2$  formed in the rumen is converted into  $\text{CH}_4$  (Mills et al., 2001). Besides methanogenesis,  $\text{H}_2$  and  $\text{CO}_2$  can be converted to acetate by acetogens, which are also present in the rumen environment (Johnson & Johnson, 1995; Demeyer & Fievez, 2000; Moss et al., 2000). Based on thermodynamics, Kohn & Boston (2000) argued that under normal fermentation conditions in the rumen, methanogenesis is feasible (reduction potential below -0.3 Volt), but for acetogenesis to occur more reducing conditions are required. This means that acetogenesis does not play an important role in the rumen hydrogen balance and the quantities of  $\text{CH}_4$  produced.

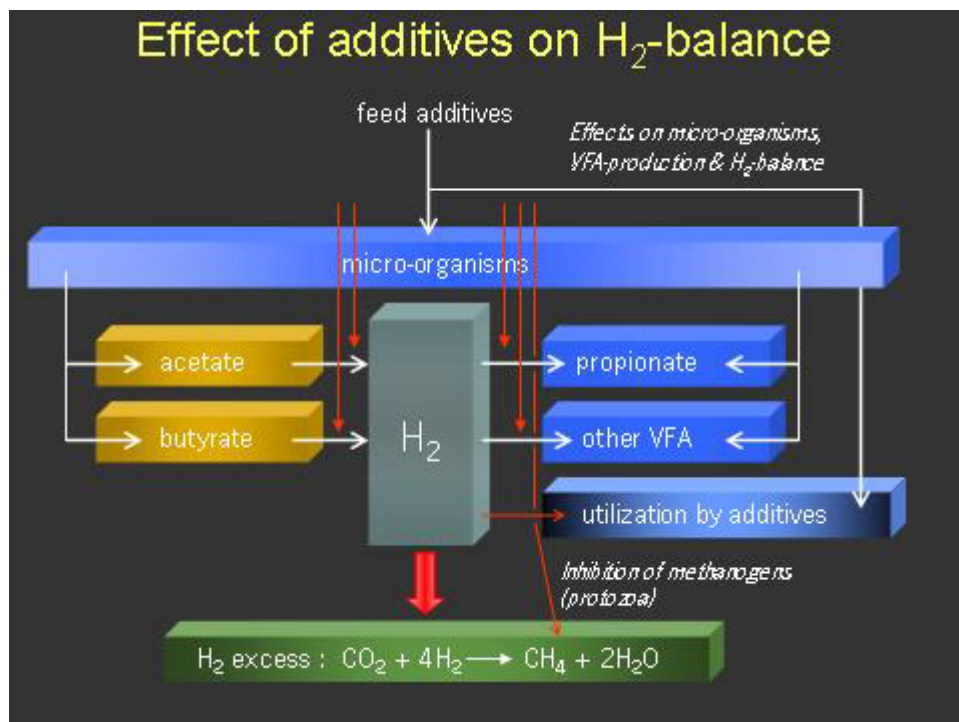
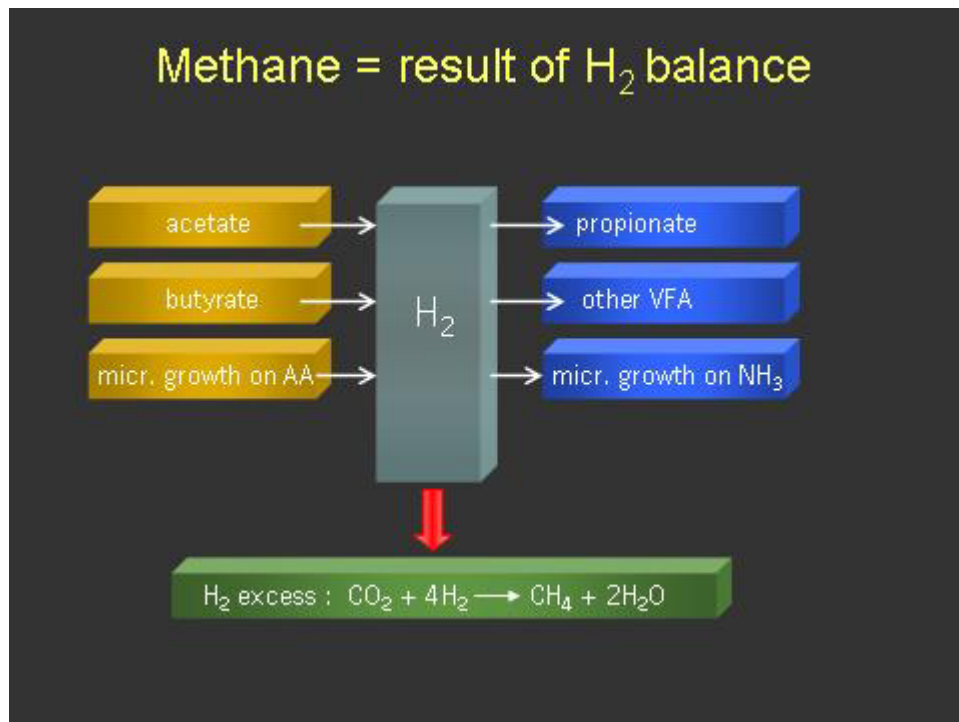
## 2.2 Contribution of hindgut fermentation

In addition to that in the rumen, also fermentation in the hindgut contributes to enteric  $\text{CH}_4$  production. This contribution appears generally to be less than 10% and slightly lower than the contribution of the hindgut to the digestion of organic matter (Kebreab et al., 2006b). The opposite would be expected because hindgut fermentation yields relatively less propionate and, as will be explained in section 1.3, hence more  $\text{H}_2$  is formed than in the rumen. Apparently, the stoichiometry of VFA production differs between rumen and hindgut. A possible explanation is a higher contribution of acetogenesis to remove the  $\text{H}_2$ , because in comparison to the rumen conditions, fermentation conditions in the hindgut may be more favourable for acetogenesis than for methanogenesis (De Graeve & Demeyer, 1988). With acetogenesis, acetate rather than  $\text{CH}_4$  is formed from  $\text{CO}_2$  and  $\text{H}_2$  (Kohn & Boston, 2000; Offner & Sauvant, 2006). Hence, removal of  $\text{H}_2$  by acetogenesis reduces  $\text{CH}_4$  yield.

## 2.3 VFA yield, hydrogen balance and $\text{CH}_4$ yield

Together with the production of VFA, and depending on the type of VFA,  $\text{H}_2$  is either generated or utilized (Figure 2). With acetate and butyrate production,  $\text{H}_2$  is produced, whereas with propionate and valerate production,  $\text{H}_2$  is utilized. Acetate is the main VFA resulting from rumen fermentation of fibre, starch, sugars and protein (Bannink et al., 2006a), and therefore a net excess of  $\text{H}_2$  is produced in the rumen. Although some other sinks of  $\text{H}_2$  can be identified (microbial synthesis with  $\text{NH}_3$  as the N source and the biohydrogenation of unsaturated fatty acids), the type of VFA produced is the major determinant of the amount of  $\text{H}_2$  produced. The specific addition of other  $\text{H}_2$  sinks to the diet may further affect the size of the  $\text{H}_2$  excess, and hence  $\text{CH}_4$  yield. Reversely, the addition of compounds such as ionophores, which affect the viability of specific classes of micro-organisms (Chen et al., 1977), may force a shift in the rumen fermentation patterns towards more propionate and a lower  $\text{H}_2$  excess (Kohn & Boston, 2000; Offner & Sauvant, 2006). It is generally assumed that the excess  $\text{H}_2$  is almost completely converted into  $\text{CH}_4$  by methanogens. As a result, the partial gas pressure of  $\text{H}_2$  remains low under normal fermentation conditions (Moate et al., 1997).

**Figure 2** Schematic representation of the effect of VFA profile on the  $H_2$  excess (red arrow) and subsequent  $CH_4$  yield (left), and of the impact of feed additives on this  $H_2$  excess (right). Adapted from Kebreab et al. (2006a)



## 2.4 Factors affecting type of VFA formed

The main part of degraded substrate becomes fermented into VFA in order to generate energy for the microbes. The profile of VFA produced depends on the type of substrate, the type of fermenting micro-organism (Murphy et al., 1982; Argyle & Baldwin, 1988; Pitt et al., 1996; Dijkstra & Bannink, 2000; Friggens et al., 1998; Bannink et al., 2006a), and the fermentation conditions in the rumen (Pitt et al., 1996; Bannink & Dijkstra, 2005; Kohn & Boston, 2000). The influencing factors and the approaches adopted to analyse their effect on rumen VFA have recently been reviewed by Bannink & Tamminga (2005) and Dijkstra et al. (2006b). Further details and approaches to quantify individual types of VFA will be discussed in section 2.5.

## 2.5 Factors influencing CH<sub>4</sub> yield

Factors that can be identified as influencing CH<sub>4</sub> yield include dietary characteristics as well as the fermentation conditions in the rumen. Important dietary characteristics are daily feed intake and the resulting rumen fill (1), the proportion of concentrates in dietary dry matter (2), and the composition and the rate and extent of degradation of individual feed fractions (the types of carbohydrate and protein) in dietary dry matter (3). Among important fermentation conditions are acidity (pH) of rumen fluid (1), the presence of unsaturated long chain fatty acids (2), the composition of the microbial population within the rumen (3), the dynamics of the passage of particles, fluid and the microbial population (4), the inflow of saliva (5) and the absorption capacity of the rumen wall. The combined effect of both types of factors is represented in conditions that characterize the ruminant, such as production level (1), stage of lactation (or other physiological conditions like pregnancy) (2), and management related interventions (3) such as grazing regime, feeding regime (diet supplementation, concentrate feeding, use of by-products like beet pulp, and protein sources), housing and milking.

Methane yield can thus be related to many and different categories of factors. However, these factors are often interrelated and so are their effects on CH<sub>4</sub> yield in the rumen. This complicates the use of such factors to predict the course of fermentation in the rumen, the extent of organic matter digestion and the productive response of the ruminant. Due to these interrelationships, the contribution of a single feed component or type of carbohydrate to CH<sub>4</sub> yield is not necessarily constant (Smink et al., 2003), but may vary with a change of the dietary characteristics and the fermentation conditions in the rumen (Mills et al., 2001; Bannink et al., 2005a).

Changing the level of feed intake, the dietary characteristics or the fermentation conditions in the rumen affect the extent of substrate degradation by micro-organisms and the efficiency of microbial synthesis. As a consequence, amounts of microbial matter as well as undegraded feed substrate flowing out of the rumen to the small intestine change. For example, a higher fractional outflow rate of feed substrate and their associated microbes leads to less degraded substrate but, because of its reduced rumen residence time, it may be accompanied with an increased efficiency of microbial synthesis (Dijkstra et al., 1998 & 2005). Because of the multiple factors that may have changed simultaneously and have affected rumen fermentation and hence CH<sub>4</sub> yield, the effect of nutritional measures on VFA and CH<sub>4</sub> production may be difficult to predict and interpret. The observed effect of a nutritional intervention on CH<sub>4</sub> yield is therefore strongly confounded with the concomitant changes brought about in these factors. Some of the principal factors affecting rumen function and CH<sub>4</sub> production will be discussed below.

### 2.5.1 Feed Intake

Changes in dry matter intake not only affect the amount of substrate available for microbial degradation, but it also changes fermentation conditions and the size of the microbial population. For example, the fate of ingested starch changes with changes in the amount of dry matter ingested, as increased intake levels will lead to a proportionally higher amount of starch digested in the small intestine rather than fermented in the rumen. Aspects which need to be considered are the storage of starch by micro-organisms with increasing concentrations in the rumen, or an altered passage rate which alters the time available for microbial degradation. Effects of feed intake level, however, are strongly confounded with most of the factors discussed below in the paragraphs 2.5.3, 2.5.4 and 2.5.5. Almost all models that predict CH<sub>4</sub> production by ruminants require daily feed intake or a closely related variable as an input.



### 2.5.2 *Intrinsic degradation characteristics*

Microbial degradation of substrates in the rumen depends primarily on intrinsic characteristics that determine the susceptibility of the substrate to be attacked, degraded and utilized by micro-organisms. These characteristics differ between types of substrate and between types of feedstuffs that are used as dietary components. Obviously, intrinsic characteristics are important determinants of substrate degradation and utilization by micro-organisms, VFA production and the concomitant CH<sub>4</sub> yield. Passage rate of substrate together with their intrinsic degradation characteristics determines the fraction of a substrate that becomes degraded in the rumen or escapes to the small intestine. In present day dairy nutrition, feedstuffs are often selected to increase the quantity of starch and protein escaping rumen fermentation, hence contributing to the nutrient supply of the cow without generating VFA and CH<sub>4</sub>. Hence, a higher passage rate due to a higher feed intake level as well as a less degradable substrate may both increase the escape of substrate and lead to a decrease in CH<sub>4</sub> yield.

### 2.5.3 *Type of substrate fermented and type of diet*

Different types of fermented carbohydrate give different profiles of VFA production and hence CH<sub>4</sub> yield (Murphy et al., 1982; Argyle & Baldwin, 1988; Pitt et al., 1997; Friggens et al., 1998; Bannink et al., 2006a). With higher levels of milk production, basal rations of dairy cows are supplemented with concentrates. Independent of the effect of fluid acidity, an analysis of VFA profiles showed about 25 and 15% lower CH<sub>4</sub> yields for fermented sugars and starch, respectively, on concentrate-rich diets compared to forage-rich diets (Bannink & Dijkstra, 2005).

### 2.5.4 *Fermentation rate and fluid acidity*

The acidity of rumen fluid (pH) influences rumen fermentation in two ways. Firstly, pH values lower than 6.2 appear to reduce the activity of fibrolytic micro-organisms degrading cell walls (Argyle & Baldwin, 1988; Dijkstra et al., 1992). Therefore, pH determines cell wall degradability and its contribution to microbial growth, and VFA and CH<sub>4</sub> yields. Secondly, pH determines the profile of VFA produced (separate from the type of substrate and the type of diet as was discussed in section 1.5.3). An increased rate of substrate fermentation, as a result of an increased feed intake or due to large concentrate meals, leads to increased rates of VFA production, to higher VFA concentrations and a more acidic rumen fluid. As a result, also the profile of VFA shifts towards a propionate oriented fermentation (Dijkstra et al., 1994). This will cause a lower H<sub>2</sub> excess and lower CH<sub>4</sub> yield. In an analysis of *in vivo* data on rumen fermentation, a decrease of the pH of the rumen fluid from 6.5 to 5.5 was estimated to lead to about 15% less CH<sub>4</sub> produced from both fermented sugars and starch (Bannink et al., 2005a & 2005b). Not only VFA concentrations determine rumen fluid pH (Baldwin, 1995; Mills et al., 2001), but also the buffering capacity of saliva flowing into the rumen (Pitt et al., 1996; Imamidoost & Cant, 2006; Bannink & Dijkstra, 2006). Buffering of rumen contents is enhanced by the inclusion in the diet of ingredients that stimulate rumination (such as straw), by feeding mixed rations, by frequent feeding (particularly of concentrates), and by preparing the rumen wall for strong increases in concentrate intake during the first weeks of lactation. If buffering processes are stimulated at the same time, an increased feed intake rate does not necessarily lead to a strong acidification of rumen fluid.

## 2.6 Conclusions

Three basic aspects need to be considered in any quantitative analysis of CH<sub>4</sub> yield in the gastrointestinal tract: 1. the quantity of organic matter fermented, 2. the partition of this organic matter into that incorporated into microbial matter and that used to generate energy growth and which yields VFA, and 3. the type of VFA formed. Many factors are known to affect each of these aspects. Dietary changes or changes in farm management may change several of these factors simultaneously, which complicates evaluation of the effectiveness of mitigation options apparent from measured CH<sub>4</sub> losses. The level of understanding of observed variation in CH<sub>4</sub> losses determines to what level of detail factors are to be investigated or to be represented in quantitative analyses.

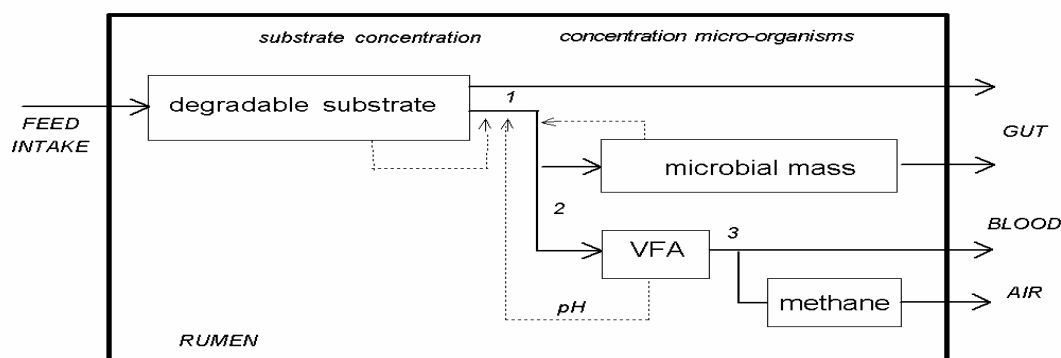
### 3 CH<sub>4</sub> prediction models

Dairy cows are kept under widely different conditions. Besides that, they possess a very complicated digestive system. Both aspects prevent the development of a set of simple rules to estimate their CH<sub>4</sub> losses. Information on CH<sub>4</sub> losses and its sources of variation is important to make reliable national inventories, but also to evaluate and prioritise research proposals for their effectiveness and feasibility. Hence, models are needed that predict CH<sub>4</sub> losses under a wide range of different conditions.

#### 3.1 Choice of model

It is extremely important to consider beforehand the specific aim a model has been developed for and the approach that was used to develop the model, before deciding on the applicability of a model and on a comparison among alternative models (Thornley and France, 2007). Several models that predict enteric CH<sub>4</sub> production by ruminants have been published. They vary in complexity and level of aggregation and range from rather simple static, empirical models to complicated dynamic, mechanistic models and all forms in between. In general, simple models are less suitable to evaluate the effects of new measures (not tested in the studies underlying the original data), of new influencing factors (not yet included in the list of explaining variables included in the model), or of interactions between various variables already included in the model. Already a decade ago, Johnson & Johnson (1995) concluded that simple empirical equations based on feed characteristics can not be expected to predict CH<sub>4</sub> accurately under various production conditions. To be able to do this, the model should represent the mechanisms at lower levels of organisation as indicated in Figure 3.

**Figure 3** Scheme of interactions between substrate, micro-organisms, and VFA and CH<sub>4</sub> yield in the rumen. Boxes indicate rumen pool sizes, solid arrows indicate flows between pool sizes, and dashed arrows indicate the influence of the microbial and VFA pool size on the rate of substrate conversion into microbial mass or VFA. The three principal factors governing CH<sub>4</sub> yield are indicated by the numbers 1, degradation of organic matter, 2, efficiency of microbial growth, and 3, type of VFA produced



More detailed models of rumen function represent the effects of intrinsic degradation and passage characteristics (Baldwin et al., 1987; Dijkstra et al., 1992; Pitt et al., 1996; Mills et al., 2001; Kebreab et al., 2004; Danfær et al., 2006). As yet, only a few mechanistic models are capable to represent substrate degradation as a function of the effective concentrations of substrate as well as of degrading classes of micro-organisms co-existing in the rumen (Baldwin et al., 1987; Dijkstra et al., 1992; Mills et al., 2001; Kebreab et al., 2004). Such an approach takes into account the interactions between feedstuffs that govern nutrient degradation, including the effect of rapidly degradable carbohydrates on degradation of fibre, or the effect of limiting amounts of nitrogen on the degradation of substrate.

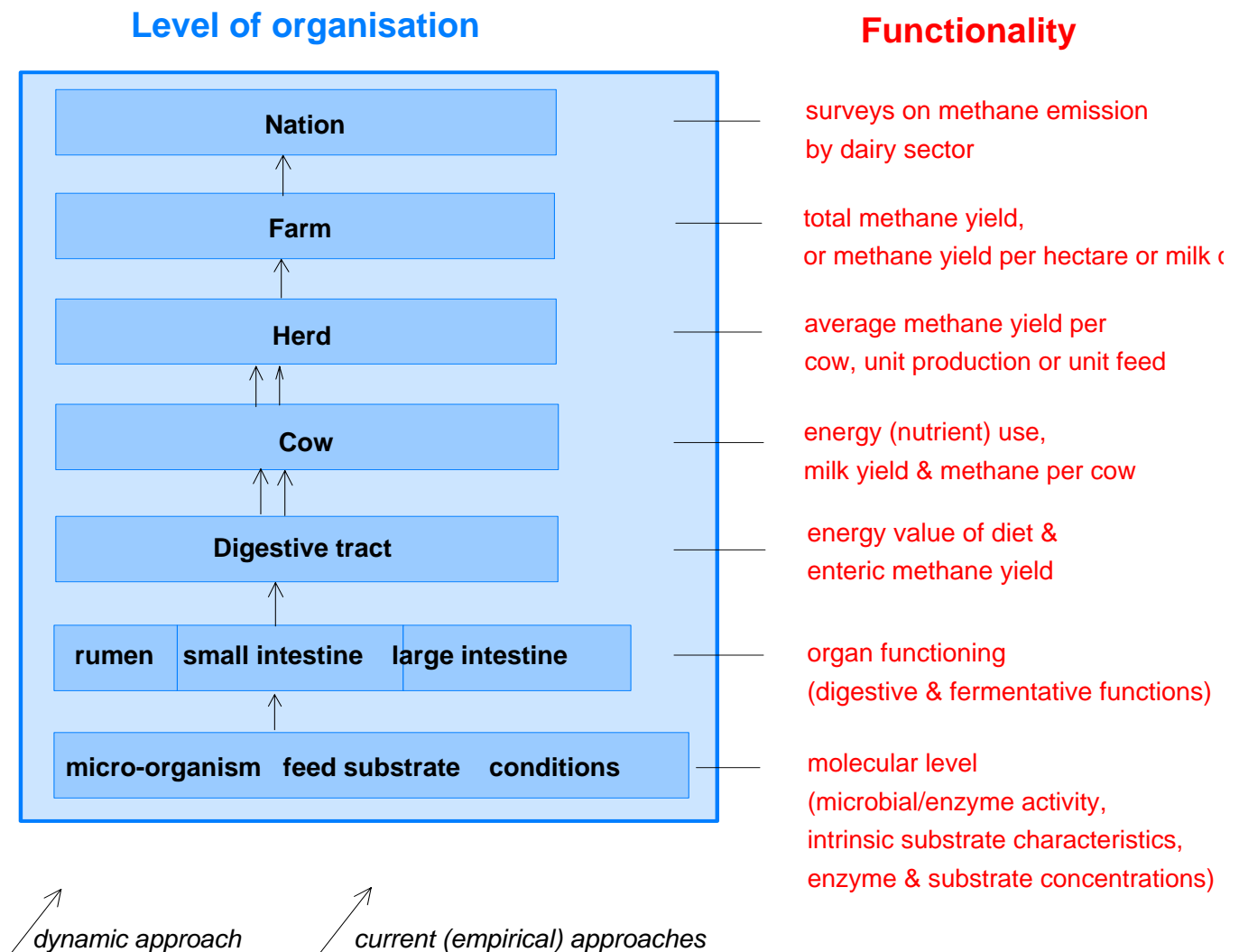
Van Laar & Van Straalen (2004) stated that one cannot decide in advance that adopting a more mechanistic approach to predict CH<sub>4</sub> is more useful than a rather empirical approach. However, an empirical model can only be applied within the range of data used in their development and, as a consequence, is by definition unsuitable to evaluate new feeds or feeding strategies that were not included in its original database (Thornley and France, 2007). Mechanistic models can be used to answer 'what-if' questions (e.g., what development in CH<sub>4</sub> production

will occur upon a change in diet composition) and are the preferred choice to identify mitigation options. Despite a usually observed large effect of the level of feed intake on  $\text{CH}_4$  emission, Van Straalen (2005) indicates that the model of Van Laar & Van Straalen (2004) predicts  $\text{CH}_4$  by taking only into account the dietary composition and degradation characteristics. The model was built using similar principles as those used by Pitt et al. (1996) but differs in details such as the representation of VFA formation.

The quantification of the type of VFA produced should be an important element of any mechanistic model aimed to explain  $\text{CH}_4$  production, since the type of VFA has a major effect on amount of  $\text{CH}_4$  produced, as explained previously. Several attempts have been made to quantify VFA yield from the substrates fermented in the rumen as recently reviewed by Dijkstra et al. (2007). In a comparative evaluation study, Bannink and Tamminga (2005) found large differences among the different representations of VFA yield. It appears that with a decline of rumen pH the yield of propionate from fermented sugars or starch increases strongly (about 100% and 50% more propionate from sugars and starch fermented into VFA, respectively, on roughage-rich as well as on concentrate-rich diets; Bannink & Dijkstra, 2005). The lower pH may have been caused by an increased rumen fill, but also result from an increase of the proportion of concentrates in dietary dry matter, and in both cases relatively more propionate is formed. In agreement with this empirical observation, Kohn & Boston (2000) formulated an explanation for this phenomenon from a thermodynamic perspective. With an increased rate of substrate fermentation and a higher partial gas pressure of  $\text{H}_2$  in the rumen, the efficiency of  $\text{CH}_4$  production declines as well as that of acetate formation. As a result of these changed conditions, propionate formation becomes relatively more favourable. Such conditions are strongly correlated to high rates of fermentation and more acidic conditions in the rumen (Bannink & Dijkstra, 2005). Empirical models do not include the large variation in VFA profile that results from the factors described above.

Some of the empirical models were recently reviewed and evaluated by Veen (2000), Mills et al. (2003), Van Zijderveld & Van Straalen (2004), Van Straalen (2005) and Kebreab et al. (2006a). The prediction accuracy of static empirical models and dynamic mechanistic models have also been evaluated against each other. Both Benchaar et al. (1998) and Kebreab et al. (2006a) demonstrated that the dynamic mechanistic models always perform among the best. Concerning the static empirical models, not always a clearly superior model is revealed, or different models appear superior in different evaluation studies. In some cases, a static empirical model seems to compare with the performance of the dynamic mechanistic models. This does not justify the conclusion that static empirical models perform just as well as dynamic mechanistic models. In contrast to the former category of models, the latter category is better capable to evaluate the consequences on details of rumen function, interactions among feed components, and on other aspects of ruminant production (diet digestibility, milk yields, excretion and manure composition, ammonia emission, etc.).

**Figure 4** Differences in the level of organization that may be chosen to explain variation in enteric CH<sub>4</sub> production in ruminants. Adapted from Bannink et al. (2005b)



A further example is the good performance of the static non-linear model of Mills et al. (2003) in a model comparison study. The model describes a nonlinear relationship between CH<sub>4</sub> yield and the ratio of starch to acid detergent fibre (ADF) content in dietary dry matter. This straightforward effect of carbohydrate type on CH<sub>4</sub> yield could however not be reproduced in a simulated variation in CH<sub>4</sub> yield among practical farms (Bannink et al., 2005a). This contradictory finding also illustrates the difference among static empirical models and dynamic mechanistic models in the type and number of effects that can be taken into account by the model (Bannink & Dijkstra, 2005).

The examples above demonstrate that following an integrated approach with dynamic mechanistic models has added value over static empirical approaches. These models take into account the underlying mechanisms, which are responsible for degradation of organic matter by micro-organisms and for the fermentation of substrates to VFA and yield of CH<sub>4</sub> as a result of methanogens removing the H<sub>2</sub> excess.

The general characteristics of the various types of CH<sub>4</sub> prediction models that have been published, and reviewed previously, are listed in Table 1. In the following, the applicability of these models will be discussed.

### 3.2 Static empirical models

Many of the factors discussed in section 2.5 have been included in static empirical models. All linear static empirical models listed in Table 1 either consider dry matter intake, or the combination of milk yield and body weight to explain CH<sub>4</sub> yield. Additional variables in the models that are related to CH<sub>4</sub> yield are digestibility, carbohydrate type and proportion of roughage in dietary dry matter. Digestibility in combination with feed intake indicates the amount of organic matter fermented in the rumen. Taking into account the type of digested carbohydrate reflects to some extent the type of VFA produced in the rumen and the excess H<sub>2</sub> converted into CH<sub>4</sub>. Except for the site of digestion (rumen or intestine), the most important aspects therefore seem to have been represented to some crude extent in these empirical equations.

Although these models often appear to give a reasonable description of the measured variation in CH<sub>4</sub> emission by ruminants, their general application is problematic (Benchaar et al., 1998). These models appear not to guarantee prediction accuracy. Recently, Van Straalen (2005) performed an evaluation of the model of IPCC (1996), Mills et al. (2003), Moe & Tyrell (1979) and Yan et al. (2000) against data from respiration trials in Lelystad (the same data that were analysed by Bruinenberg et al., 2002). From this evaluation it was concluded, under the assumption of a fixed animal production level of three times maintenance, that the model of Yan et al. (2000) was most applicable to predict CH<sub>4</sub> emission on Dutch dairy farms (participants of project Cows & Opportunities). The model of Yan et al (2000) takes into account digestible energy intake and the fraction of silage ADF in total dietary ADF. However, the model does not take into account the type of carbohydrate digested and the site of digestion (rumen digestion contributes to CH<sub>4</sub> emission whereas intestinal digestion does not). The model of Moe & Tyrrell (1979) takes into account the type of carbohydrate digested as well as the amounts digested, but also this model does not distinguish between digestion in the rumen and the intestine. Benchaar et al. (1998), and more recently Kebreab et al. (2006a), evaluated the static models of Blaxter & Clapperton (1965) and of Moe & Tyrrell (1979) and these studies indicated that predictions by these equations should not be expected to become very accurate in general (prediction error of 20 to 30% of the observed mean were established).

Although Kebreab et al. (2006a) found the non-linear approach of Mills et al. (2003) to improve prediction accuracy, Van Straalen (2005) disqualified this model in favour of the model of Yan et al. (2000). But, no details of the evaluation results were described. These examples of evaluation studies do illustrate, however, the contradictory results that may be obtained when evaluating static empirical models on different data sets. More importantly, such model evaluations do not give a clue about the precise cause of these conflicting results among various studies. One possible cause may be the general nature of the explanatory variables used. Digestibility of dietary dry matter and the type of carbohydrate digested in the rumen are estimated from feed evaluation data of individual dietary components. Such data presume that the characteristics of dietary components are fixed and additive, and may hence be weighted according to the contribution of the component to dietary dry matter. In reality, however, characteristics vary with the level of feed intake and with the fermentation conditions in the rumen (volume, outflow rates, acidity). The static models listed in Table 1 do not accommodate for this type of variation in digestibility characteristics and in the type of carbohydrate digested in the rumen.

In conclusion, many attempts have been made to introduce various nutritional factors in static models as explaining variables for CH<sub>4</sub> emission. Although these factors are closely related to the process of CH<sub>4</sub> formation in the rumen, their use for predictive purposes is considered to be too general to obtain sufficient accuracy or to obtain a satisfying explanation of the variation observed among nutritional treatments.

### 3.3 Dynamic empirical models

In order to improve the explanation of rumen function and CH<sub>4</sub> yield, more dynamic approaches have been adopted. Although often also a more mechanistic approach is claimed, many dynamic models still have a rather empirical and static basis. Examples of such models are that of Russell et al. (1992), of Lescoat & Sauvant (1995), of Pitt et al. (1996), of Van Laar & Van Straalen (2004) and of Danfær et al. (2006). The reason why these models are qualified as being of an empirical and static nature is that microbial activity is fully determined by the intrinsic degradation characteristics of feed substrate and substrate outflow dynamics. Substrate degradation was not represented as a function of the quantity of microbial matter present in the rumen (Bannink et al., 2006b). Moreover, these dynamic models usually do not include interactions between various substrates that determine extent of degradation or type of VFA formed. Although all of the models are often referred to as dynamic and mechanistic, the fraction of substrate that is degraded could just as well have been calculated by the ratio calculation of  $k_d/(k_d+k_p)$ , with  $k_d$  and  $k_p$  as the fractional degradation rate and the fractional outflow

rate, respectively. Exactly the same type of calculations are used in the DVE/OEB-system (Tamminga et al., 1994) and applied in the current practice of feed evaluation (CVB, 2000), and have been applied by Smink et al. (2003).

Without using a rumen model, Smink et al. (2004) used an approach similar to the type of calculations in the models of Van Laar & Van Straalen (2004) treating  $\text{CH}_4$  yield of individual feedstuffs as a constant value depending on degradation and passage characteristics.

Further, microbial growth is represented in a rather empirical manner without a representation of the processes involved. For instance, in the model of Russell et al. (1992) and Pitt et al. (1996) the double reciprocal Pirt equation has been applied to calculate microbial growth assuming that fractional rates of microbial growth and fractional rate of substrate degradation are equal. An identical approach was adopted in the  $\text{CH}_4$  prediction model of Van Laar & Van Straalen (2004). Dijkstra et al. (2002) argued, however, that this approach is biologically impossible and not likely to be a good approximation of microbial growth under many circumstances. More recently, Dijkstra et al. (2005) showed that efficiency of microbial growth does not depend on rate of degradation, but rather on rate of outflow of the rumen. Actual results of experiments confirm such relationships (e.g., Oba & Allen, 2003). This type of dependency was not represented in these models.

In this category of models, other aspects of fermentation have also been represented in a highly empirical way. For example, the effect of pH on the activity of cell wall degrading micro-organisms is rarely represented. The production of individual types of VFA is often estimated from results of *in vitro* trials of feed substrate incubated with rumen fluid under highly diluted, strongly buffered and standardized conditions. Both Pitt et al. (1996) and Van Laar & Van Straalen (2004) derived equations for the prediction of VFA yields from the results of *in vitro* trials. Although the origin of the data used by the latter authors remains unknown, they do mention large differences between two laboratories in the type of VFA retrieved from *in vitro* incubations of identical feed material. Such conflicting findings demonstrate that differences among research groups in the *in vitro* measurement protocol easily leads to different estimates of VFA yield. A typical example of the problems involved in using *in vitro* VFA stoichiometry is that the level of feed intake of a constant dietary composition will give widely different VFA profiles *in vivo*, which cannot be mimicked *in vitro*. This also indicates that care should be taken to estimate VFA yield from *in vitro* data as recently discussed by Dijkstra et al. (2006b). Accurate representation of the type of VFA yield is however essential for an accurate prediction of  $\text{CH}_4$  yield (Benchaar et al., 1998; Mills et al., 2001; Bannink et al., 2005a & 2005b; Kebreab et al., 2006a).

In this respect, it is interesting to note that the dynamic empirical Van Laar & Van Straalen (2004) model predicted the methane production measured in respiration chambers in Wageningen on three diets (control, high in fat, high in maize silage) with less accuracy than the static empirical IPCC model. The squared error was 1.01 and 0.27 (g methane/kg milk)<sup>2</sup> at an observed average of 12.7 g methane/kg milk.

In conclusion, many dynamic models of rumen fermentation focus at the representation of the dynamics of substrate degradation and outflow. Although more details are taken into account concerning type of carbohydrate degraded and presumed efficiencies of microbial growth, from a modelling perspective these models must still be considered as rather empirical. They still adopt a rather static representation of substrate degradation and microbial growth (which could just as well have been simplified to static equations). Also, applicability of estimated VFA yields for *in vivo* conditions is questionable for most models and should at least be documented and evaluated before applying these models to predict  $\text{CH}_4$ .

### 3.4 Dynamic mechanistic models

A more integrative approach was adopted in the model of Baldwin et al. (1987) and more recently by Mills et al. (2001) based on the model of Dijkstra et al. (1992). Recently, the latter model has been adapted by Bannink et al. (2005a) again and applied by Dijkstra et al. (2006). In contrast to the previous empirical mechanistic models, these models take into account the consequences of feed intake level on the effective concentrations of substrates and different classes of micro-organisms that co-exist in the rumen (Bannink et al., 2006b), both of which are determinant factors for the extent of substrate degradation. Mills et al. (2001) demonstrated that with an increase of feed intake from 10 to 25 kg dry matter per day the yield of  $\text{CH}_4$ , as a percentage of GEI, declines with almost 10%. In practice the yearly average feed intake in high producing dairy cows in The Netherlands varies between 20 and 24 kg of DM, and hence the potential to reduce  $\text{CH}_4$  emission by an increased feed intake and production level remains limited. Animals of lower genetic merit or animals at the end of lactation or in the dry period have a much lower feed intake, especially when fed to official CVB guidelines (as low as 10 kg DM/day). Recently, effects of fluid acidity on the VFA profile were included in the model (Bannink et al., 2005a;

Dijkstra et al., 2006), resulting in a further reduction of CH<sub>4</sub> yield with increasing acidity of rumen fluid and with an increasing proportion of concentrates in dietary dry matter.

Only the models of Baldwin et al. (1987) and Dijkstra et al. (1992) take a full dynamic approach to represent the dynamics of microbial growth (Table 1). The model of Baldwin et al. (1987), and subsequent adaptations of this model (Baldwin, 1995), contains details on the process of feed particle size reduction, but it does not take into account the intrinsic degradation characteristics of the dietary components. Despite the importance of the mechanisms of particle size reduction, of microbial attachment to these particles and of the impact of rumination on these processes, their representation appears insufficient to fully explain the variation in degradation characteristics observed for various dietary ingredients. For this reason, all other dynamic rumen models take into account the intrinsic degradation characteristics of dietary ingredients, including the dynamic mechanistic model of Dijkstra et al. (1992), and subsequent adaptations of that model (Mills et al., 2001; Kebreab et al., 2004; Dijkstra et al., 2006a). Generally, the intrinsic degradation characteristics are considered most important to explain rumen substrate degradation. Although models have been developed which represent detailed aspects of the thermodynamics of microbial metabolism in the rumen (Kohn & Boston, 2000; Offner & Sauvant, 2006; Table 1) these models do not seem applicable yet to predict rumen function in response to a wide range of nutritional factors.

Several additional aspects were represented in the dynamic mechanistic models of Baldwin et al. (1987) and Dijkstra et al. (1992); firstly, the impact of rumen acidity on the degradation of cell wall carbohydrates (Argyle & Baldwin, 1989; Dijkstra et al., 1992) and on the type of VFA produced (Argyle & Baldwin, 1988; Bannink & Dijkstra, 2005); secondly, an increasing storage of microbial starch with an increasing supply of rapidly fermentable carbohydrates (Dijkstra et al., 1992). The latter feature, in combination with the effect of the intrinsic starch degradation characteristics and the rumen concentration of microbial matter, allows much more variation in the prediction of the fraction of starch escaping rumen fermentation. In a direct comparison of the models of Baldwin et al., (1987) and Dijkstra et al. (1992), the latter model was judged to perform best in this respect (Bannink et al., 1997a). Further, only the model of Dijkstra et al. (1992) considers the interaction among several classes of micro-organisms (sugar and starch utilizing bacteria, cell wall utilizing bacteria and protozoa utilizing feed substrates but predating on bacteria as well), enabling the representation of intra-rumen recycling of microbial matter. This model feature is important in relation to the efficiency of synthesis of microbial matter flowing out of the rumen.

With regard to the prediction of CH<sub>4</sub>, Benchaar et al. (1998) made a direct comparison between both models and also a comparison against static empirical equations. The Dijkstra et al. (1992) model performed best. Mills et al. (2001) further developed the model of Dijkstra et al. (1992) and included new estimates of VFA yield which Bannink et al. (2000; 2006a) had derived from *in vivo* data. Recently, these VFA yields were re-estimated and made dependent on rumen acidity (Bannink & Dijkstra, 2006). The effect of acidity of rumen fluid was added as an explanatory variable in regression studies of *in vivo* data on rumen fermentation in lactating cows (Bannink & Dijkstra, 2005). The resulting new values were applied by Bannink et al. (2005a & 2005b) in an evaluation of CH<sub>4</sub> emission on several farms in practice, and by Dijkstra et al. (2006) in evaluating the development of CH<sub>4</sub> emission by dairy cows in The Netherlands during the years 1990 to 2003. The latter version of the model was applied by Bannink et al. (2005a & 2005b), Smink et al. (2005) and Dijkstra et al. (2006) to predict CH<sub>4</sub> emission. Momentarily, this model is extended with a representation of the impact of saturated and unsaturated fatty acids on microbial metabolism. Further, other aspects of rumen function have recently been added, such as equations which allow the prediction of excretion with urine and faeces (Reijs et al., 2006).

In conclusion, the model of Dijkstra et al. (1992), and subsequent adaptations of this model, are of a highly mechanistic nature and represent the influence of many key factors identified in literature. For this reason, the model is considered a useful research instrument to study the effectiveness of nutritional measures to reduce CH<sub>4</sub> yield by cattle. The simulation results using this model are momentarily included in the Dutch National Inventory Reports of greenhouse gas emissions. Besides the emission of CH<sub>4</sub>, it has recently been applied as well to generate useful information on other consequences of cattle nutrition such as excretion rates, excreta composition and consequences for ammonia emission (Reijs et al., 2006).

### 3.5 Model elements lacking

Various CH<sub>4</sub> prediction models of various levels of complexity were discussed. All these models essentially lack the representation of effects of feed additives on microbial fermentation, on VFA and CH<sub>4</sub> yield, and on characteristics of animal performance (feed digestibility, metabolizable energy, milk yield or growth). Some

additives, such as long chain fatty acids, have a detrimental effect on the activity of protozoa and, hence, on methanogens closely associated with them. But, these effects may be temporary and adaptation of rumen micro-organisms to feed additives seems to occur. Further details will be discussed further in chapter 4.

Although the model of Dijkstra et al. (1992), and its subsequent versions, considers the effect of protozoa on rumen fermentation, the model was not developed to predict the effect of feed additives on protozoal activity. Furthermore, few attempts have been published that represent the effect of long-chain fatty acids. These models still lack accuracy with respect to either the level of detail of the type of fatty acids involved (Dijkstra et al., 2000; Bannink & Dijkstra, 2007), or the robustness and the level of detail of whole rumen function represented (Moate et al., 2004). Finally, no modelling studies are known that describe the adaptation of rumen micro-organisms. All models discussed in the present study generate static predictions of CH<sub>4</sub> as a logical consequence of the nature of the model (empirical models) or because application is restricted to steady-state simulations (dynamic models).

To predict rumen adaptation, a considerable modelling effort still needs to be done. Inevitably such a modelling study needs to involve a dynamic mechanistic approach with a representation of protozoa included in the model.

### 3.6 Farm budget models

Only the modelling approach of Schils et al. (2005, 2006a & 2006b) is listed in Table 1 as an example of farm budget models. Other modelling approaches have been used to budget greenhouse gas emissions (IPCC, 1996; Oudendag & Kuikman, 2004; Kuikman et al., 2005), but they all adopt in principle the approach of fixed emission factors and therefore are considered similar to the approach of Schils et al. (2005) with respect to prediction of CH<sub>4</sub> from ruminants. This type of models typically adopts the concept of a constant CH<sub>4</sub> emission factor specific for a type of dietary ingredient, a type of diet, or a type of ruminant production system. Schils et al. (2005) estimated methane production of dairy cattle (kg yr<sup>-1</sup>) as  $50 + 0.01 \times \text{milk production}$ . For a cow producing 8000 kg milk yr<sup>-1</sup> this amounts to 130 kg CH<sub>4</sub> yr<sup>-1</sup>, some 10% lower than the methane production calculated using a large dataset as described in 4.2. Again, any dietary changes that are aimed to maintain milk production but to reduce CH<sub>4</sub> production, cannot be evaluated using this type of model. Schils et al. (2006a & 2006b) applied different CH<sub>4</sub> emission factors for grass products, corn silage and concentrates as derived by Smink et al. (2003). The main aim of these approaches is to evaluate the various sources and sinks within a farm in an integrated manner in order to generate a budget of greenhouse gases and to identify trade-offs between the individual farm compartments (herd, manure, soil, crop) and green house gases (CH<sub>4</sub>, N<sub>2</sub>O, CO<sub>2</sub>). In addition to these budget models, methodology was used to perform the life cycle assessment (LCA) of milk production which also considers other aspects of dairy farming than GHG emissions. Recently, Thomassen et al. (2007) studied the performance of organic dairy farms against conventional dairy farms. The LCA results indicated less energy use and eutrophication with organic farming, but a higher acidification potential, global warming potential and land use per kilogram of milk.

While suitable for the purpose of accounting for the various components of farming systems, these models were not developed with the aim to evaluate the effectiveness of specific nutritional measures to reduce CH<sub>4</sub> emission from enteric fermentation in cattle. Nor are they suitable to evaluate detailed effects on the level that may be of interest to the farmer and feed manufacturing industry. For these purposes more detailed models of enteric fermentation are needed. To answer questions on both the level of the ruminant and of the level of the whole farm, a combination of dynamic mechanistic models explaining the digestive and productive functions in ruminants and of a full accounting farm budget model needs to be developed and applied (Kebreab et al., 2006b).

### 3.7 Conclusions on CH<sub>4</sub> prediction models

Static empirical models appear useful for a quick appraisal of the size of changes in the level of CH<sub>4</sub> emission in ruminants that may be expected with changes in management or nutrition. In particular when information on dietary ingredients, production conditions and feed intake levels is lacking, these equations may prove to be useful. Caution is warranted however, when drawing conclusions on prediction of methane in ruminants because of the lack of accuracy and the highly empirical background of such equations. In particular, empirical models lack the biological basis necessary to evaluate mitigation strategies and cannot be used to predict changes in methane emissions outside the range they were developed for. The dynamic empirical models include more nutritional details than the static empirical models. Still, the approach remains highly empirical and of a static



nature as discussed above for substrate degradation rate. The dynamic mechanistic approaches appear most useful to assess the effectiveness of specific mitigation options (Kebreab et al., 2006a & 2006b) and these models have been shown to be more accurate than empirical models. These models are much more capable to explain causal relationships between nutrition and CH<sub>4</sub> formation. This capacity is essential when attempting to explain in detail the changes in CH<sub>4</sub> emissions in ruminants for future mitigation options. The development prediction models for the (temporary) effect of feed additives still requires a substantial modelling effort.

**Table 1** Models and variables used to explain CH<sub>4</sub> production in ruminants

Static / empirical models	Feed intake	Digestibility	Carbohydrate types	Proportion roughage	Milk yield	Body weight
<u>Linear relationships</u>						
Axelsson, 1949	X					
Blaxter & Clapperton, 1965	X	X				
Moe & Tyrrell, 1979	X	X	X			
Holter & Young, 1992 <sup>1</sup>	X	X	X	X	X	X
Kirchgessner et al., 1994	X	X	X			
Kirchgessner et al., 1995					X	X
Johnson & Ward, 1996	X	X	X			
Yan et al., 2000	X	X		X		
Corre, 2002					X	
Mills et al., 2003	X					
Hindrichsen et al., 2004	X	X	X			
Schils et al., 2006a	X		X	X		
<u>Nonlinear relationships</u>						
Mills et al., 2003	X	X	X			

Dynamic models	Represented influencing factors			Type of mechanism represented	
	Feed intake	Degradation characteristics	Passage characteristics	Microbial activity	Thermodynamics fermentation
<u>Empirical models</u>					
Lescoat & Sauvant, 1995	X	X	X		
Pitt et al., 1996	X	X	X		
Van Laar & Van Straalen, 2004	X	X	X		
Danfær et al., 2006	X	X	X		
<u>Mechanistic models</u>					
Baldwin et al., 1987	X	X	X	X	
Kohn & Boston, 2000					X
Mills et al., 2001	X	X	X	X	
Dijkstra et al., 2006 <sup>2</sup>	X	X	X	X	
Offner & Sauvant, 2006			X		X

<sup>1</sup> Multiple relationships were derived by testing various combinations (not all variables simultaneously)

<sup>2</sup> Model of Mills et al. (2001) with the representation of VFA formation derived by Bannink & Dijkstra (2006)

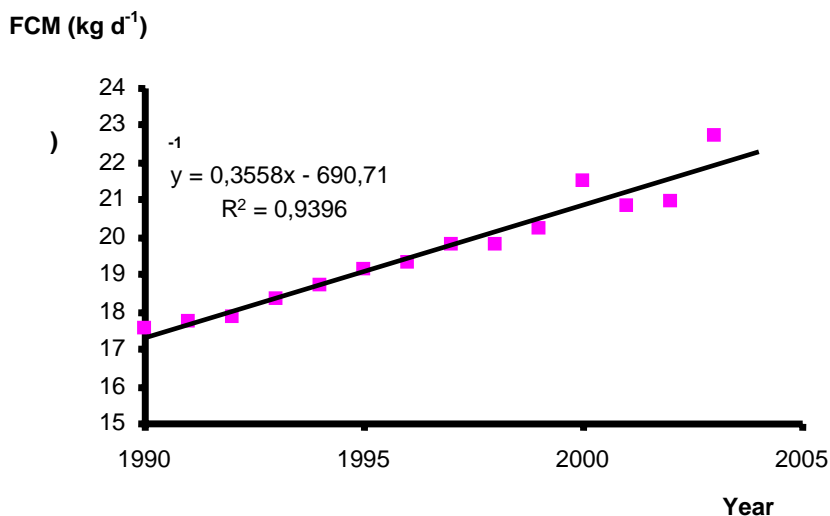
The same model was used by Smink et al. (2005) and is momentarily used for Dutch National Inventory of emission registrations

## 4 Recent and future developments in The Netherlands:

### 4.1 Recent developments

According to Veen (2000), the largest contribution to a reduction in  $\text{CH}_4$  emission is expected to come from an increased milk yield per animal, provided the milk quota system will maintain. Such an increased milk yield can be achieved by a combination of genetic selection and an optimal use of management in the field of growing and conserving forage, housing of dairy cows and milking technique, such as robotic milking. Milk production per cow in The Netherlands has increased from around 6000 kg  $\text{yr}^{-1}$  in 1990 to 7500 kg  $\text{yr}^{-1}$  in 2003 (Figure 5). With a fat content of 44 g/kg this amounts to around 8000 kg fat corrected milk (FCM)  $\text{yr}^{-1}$  or 21.9 kg FCM  $\text{d}^{-1}$  (Smink et al., 2005; Dijkstra et al., 2006). Expectations are that this increase will continue and that milk yield per cow will rise to 8800 kg FCM by 2010, the equivalent of 24.1 kg FCM  $\text{d}^{-1}$ .

**Figure 5** Milk yield per cow in The Netherlands between 1990 and 2003 (Smink et al., 2005; Dijkstra et al., 2006)



As was stated by Veen (2000), during the period 1990-2000, no research on  $\text{CH}_4$  emission by dairy cows has been conducted in the Netherlands. Because in earlier experiments  $\text{CH}_4$  emission in itself was not the subject of research and the research aimed at the efficiency with which energy was utilised, knowledge on  $\text{CH}_4$  emission in dairy cows with the present day high milk productions is lacking. According to Veen (2000), extrapolation of former results could at most give indications. He also recommended that the consequences of keeping cows in natural grasslands and alternative forms of dairy husbandry should be studied via energy balance experiments in respiration chambers. Moreover, the equation that was used to estimate national  $\text{CH}_4$  emissions following IPCC rules was considered not suitable for the Netherlands, because of the big variation in diet composition within The Netherlands (Veen, 2000). The equation was considered not applicable to reliably estimate the effects of nutrition measures, because the equation did not take into account the improved energetic efficiency of dairy cows with higher milk yields.

Several recent reports on strategies to reduce enteric  $\text{CH}_4$  emissions from dairy cows (Mills et al., 2001; Boadi et al., 2004; Dohme et al. 2004; Hindrichsen et al., 2005; Ramirez-Restrepo & Barry, 2005; Kebreab et al., 2006b) have presented figures on  $\text{CH}_4$  losses in dairy cows, covering a range of milk productions of between 10 and 40 kg  $\text{d}^{-1}$ . Regardless measuring technique (respiration calorimetry, mass balance,  $\text{SF}_6$  tracer), animal breed and diet,  $\text{CH}_4$  losses (g  $\text{CH}_4$  kg<sup>-1</sup> milk) declined almost linearly ( $R^2=0.813$ ) with 0.437 g  $\text{CH}_4$  kg<sup>-1</sup> of milk over the entire range of milk productions (figure 6). For a cow producing 8000 kg FCM this amounts to 143 kg  $\text{CH}_4$   $\text{yr}^{-1}$ .

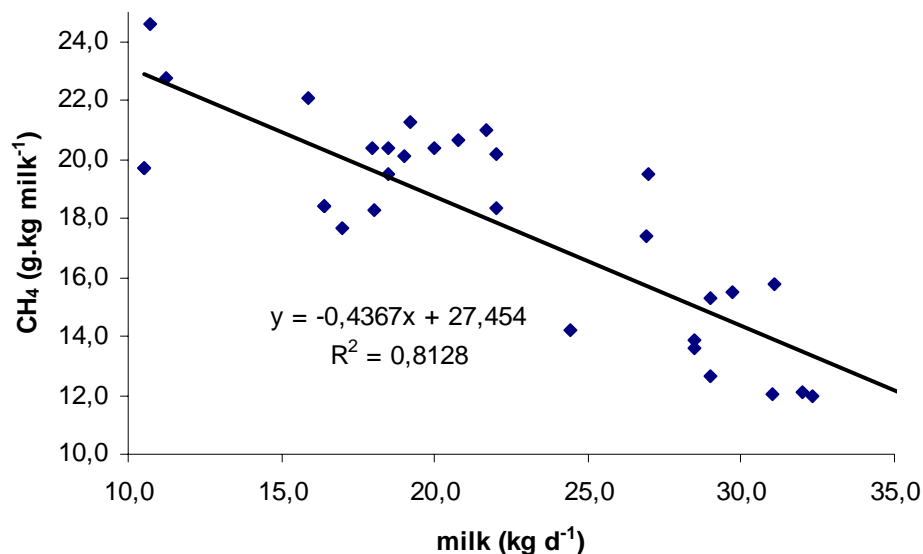
Observations reported by Van Laar & Van Straalen (2004) on methane production of dairy cattle in mid-lactation measured in Wageningen respiration chambers (viz. 12.7 g CH<sub>4</sub>.kg<sup>-1</sup> at 31 kg milk d<sup>-1</sup>) are in line with these results from literature. Similarly, more recent observations for cows in very early lactation (wk 1-9 of lactation) in Wageningen (Van Knegsel et al., 2007) (viz. 9.4 g CH<sub>4</sub>.kg<sup>-1</sup> at 40 kg milk d<sup>-1</sup>) agree with these results. Despite the similarity in levels of CH<sub>4</sub> observed in different studies, considerable variation remains among individual animals and among treatments.

Van Zijderveld & Van Straalen (2004) concluded that the IPCC estimate of 6% of the GEI for CH<sub>4</sub> production seems realistic for the Netherlands. Recent calculations by Smink et al. (2005) and Dijkstra et al. (2006) based on average intake and diet composition of dairy cattle in the Netherlands resulted in a percentage of 5.9%, hence also indicating that the estimate of IPCC is reasonable. In most surrounding countries where dairy cows have lower milk productions and where diets with a lower digestibility are fed, this estimate may be too low.

## 4.2 Future developments

Although the quota system for milk production in the EU is expected to disappear within the next decade, as long as it is in operation, expressing CH<sub>4</sub> losses per kg of milk (FCM) is an appropriate way. Increasing milk yield per cow still has scope. This will cause less feed to be lost in maintenance that does not result in (milk) production, but is still associated with CH<sub>4</sub> losses. Increasing milk production by 10% from 21.9 kg FCM d<sup>-1</sup> in 2003 to 24.1 kg.d<sup>-1</sup> (8800 kg FCM yr<sup>-1</sup>) in 2010, would reduce CH<sub>4</sub> emission per kg FCM with over 5%.

**Figure 6** Methane (CH<sub>4</sub>) losses per kg of milk in dairy cows



In the last decade, the number of young stock as proportion of the total number of dairy cows has declined and will probably decline further. When we assume that for each 10 dairy cows, 4 young animals < 1 yr and 3.25 animals > 1 yr are kept (Tamminga et al., 2004), producing 25 and 65 kg of CH<sub>4</sub> yr<sup>-1</sup> respectively (Schils et al., 2005), the CH<sub>4</sub> production from the young stock would approach 24% of that of the mature animals. Reducing this number by 8 % in 2010 would result in a further reduction of the CH<sub>4</sub> losses with 2%.

In addition to increasing milk yield per cow, there is a shift towards less grazing. This will cause a shift from animal waste excreted in meadows towards excretion in animal houses. For instance in the early nineties around 60% of the N excreted by breeding cattle (cows in milk, pregnant cows and young stock) was excreted in animal houses. In 2003 this figure had increased to almost 73% (Van der Hoek et al., 2006). This trend is expected to continue, because there is a tendency to keep lactating cows year round in the animal houses. Reducing the numbers of young stock also means a shift between the feeding of fresh grass and grass silage, towards more grass silage, with concomitant higher CH<sub>4</sub> losses. Replacement animals are fed with only marginal quantities of maize silage, so, there is little scope for a redistribution of forage types between lactating animals and young stock. The trend to keep lactating animals inside will increase CH<sub>4</sub> losses, not only direct losses (see paragraph 4.1.1.) but also indirect losses from stored manure.

This is in line with the recommendation of Veen (2000) who suggests that the main part of milk production should take place in large and highly productive dairy herds, using high quality forages and concentrates. This would create space for smaller farms in areas with an attractive and valuable landscape and in areas where grazing is the best or even the only option.

Most developments will result in a reduction of feed required for maintenance at herd level and a redistribution of feed with time and place. Apart from this redistribution, scope exists to adapt the composition of the diet in such a way that less CH<sub>4</sub> is lost per kg DM feed ingested as well as per kg of milk produced. For instance, increasing milk yield almost inevitably means feeding more concentrates in dairy diets leading to less CH<sub>4</sub> per kg of milk. However, this also causes the use of more fossil energy (Tamminga, 1996) and the emission of CO<sub>2</sub>, partly nullifying the reduced CH<sub>4</sub> emission from cows.

Nutrition and nutrition management have been suggested as promising means to reduce CH<sub>4</sub> losses as was already discussed by Veen (2000). Some specific measures will be discussed in the following sections. Alternative measures to reduce CH<sub>4</sub> losses include selection for low CH<sub>4</sub> emitters (Ulyatt & Lassey, 2001; Hegarty, 2004), and increasing fertility and longevity (Garnsworthy, 2004). Although model calculations showed that potential decreases in CH<sub>4</sub> loss with improved fertility and a reduced number of replacement stock could also reach 25%, an increased production per animal will make this difficult to achieve. The first task would be to maintain fertility, let alone increasing it.

### 4.3 In conclusion

Methane losses from dairy cows in The Netherlands do probably not exceed 6.0% of the GEI. An increase in milk yield per cow seems to be an almost autonomous development and losses per kg of milk will continue to diminish with an increased milk production per cow and a reduction of the number of cows. Increased milk yield shifts the distribution of feed to less feed for maintenance and more feed for production. This is not only true for the lactating animals, but also due to a reduction in the number of animals needed for replacement. Increased milk yield probably also means a reduction of longevity, lowering fertility, and increasing the length of the period in between two calvings. The ongoing trend to keep lactating animals inside will possibly increase direct but almost certain indirect CH<sub>4</sub> losses. Further research is needed, however, to determine the extent of change these CH<sub>4</sub> losses.

## 5 Mitigation strategies

### 5.1 Forages

According to Veen (2000), improving the digestibility of plant cell walls has limited potential, because in the Netherlands the digestibility of the basal (forage) diet of dairy cows is already high. Selection of silage maize varieties for a higher digestibility of stem and leaf was considered to still have some potential. However, he also stated that developments, mainly extensifying due to a lower N-fertilisation, might nullify the potential effects of selecting for better quality of maize (silage). He indicated that at European level there is a strive to increase the use of traditional forages and other crops that can be grown on the farm at the expense of the use of concentrates. An increasing number of dairy farmers make contracts with the owners of natural grasslands, in which grass is cut at a later stage, with a concomitant lower digestibility. In order to limit the N-surplus on their farm, dairy farmers also lower the level of N-fertilisation, which results in a lower digestibility because they cut the grass later to ensure a sufficiently high dry matter yield. In the same framework a trend is seen with dairy farmers to feed less maize silage and more whole plant silage (WPS) from other grains (triticale, barley, wheat), because these crops are more efficient in N utilisation. To a limited extent dairy farmers deliberately lower the digestibility with the aim to increase the return of C-rich compounds to the soil in order to enhance the soil structure.

Diets for lactating cows in The Netherlands contain on average 55% forage and 45% concentrates. The most important forages are fresh grass, grass silage and maize silage. Table 2 shows the average quality over the last 4 years. The results show that forage quality in The Netherlands is high with a digestibility of the OM in fresh grass of around 80% and that of grass silage and maize silage of around 75%.

**Table 2** Forage quality in The Netherlands (Blgg, Oosterbeek)

Year	Fresh grass		Grass silage			Maize silage		
	dOM	%N/DM	dOM	%N/DM	dNDF	dOM	%N/DM	dNDF
2002	81.2	3.53	75.7	2.96		75.3	1.30	49.7
2003	80.4	3.60	74.4	2.82	72.0	74.9	1.23	52.5
2004	81.7	3.60	77.6	3.06	74.1	75.3		56.7
2005	82.0	3.31	77.7	2.82	72.4	74.0		54.9

#### 5.1.1 Fresh grass

Methane production in ruminants tends to decrease with the quality of the forage fed. Quality of forages depends predominantly on maturity and less mature forage often has a higher N and lower sugar content. Maas (1987) reviewed 163 energy balance studies with dairy cows fed with over 90% of their DMI as fresh grass, performed in New Zealand (n=80) and in The Netherlands in Wageningen (n=63) and in Lelystad (n=20). The overall CH<sub>4</sub> loss was 6.33% of GEI at an average dOM of 0.768. He divided the data set into 4 groups with the N content in the OM of the grass to be between 1-2%, 2-3%, 3-4% or 4-5%. Above 3% of N (18.75% CP) in the OM, the CH<sub>4</sub> losses declined from around 6.5% of GEI at below 3% of N in the OM to 5.2% of GEI at 4.5% N in the OM of the grass. The shift in N content was associated with an increase in the digestibility of the energy (dE) from 72.2 to 74.1%. In a subsequent study Bruinenberg et al. (2002) used the same data from Wageningen and Lelystad and compared the results with 13 data from Hillsborough (Ireland). Average DCP/DM ratios in the grass were 0.238, 0.244 and 0.176 in Wageningen, Lelystad and Hillsborough with CH<sub>4</sub> losses (as % of GEI) of 5.8, 5.6 and 7.3% respectively.

Similar observations were made in grazing sheep (Murray et al., 2001). Sheep that grazed on pastures that had received 270 kg N ha<sup>-1</sup> produced significantly less CH<sub>4</sub> than animals on pastures that had received 70 kg N ha<sup>-1</sup>. The %N/OM in fresh grass in The Netherlands for the qualities presented in table 2 was on average 4.94 (s.e.0.079), suggesting a CH<sub>4</sub> loss of around 5.6% of GEI.

Expectations are that because of new manure legislation, farmers will use less inorganic N fertiliser, which may not only reduce the N/OM ratio, but may also lower the dOM and dNDF.

Increasing the digestibility of cell walls in forages has also been suggested as a means to lower CH<sub>4</sub> losses, but recent research (Taweel et al., 2005) showed that the potential to select for even high(er) cell wall digestibility in fresh grass is very limited. Grassland management and fertilization regime will have much more effect on CH<sub>4</sub> production.

### 5.1.2 Grass silage

Grass silage is usually harvested at a later stage of maturity than fresh grass, as is also shown by the results in table 2. This results in a lower dOM and a lower N content, a lower sugar content and a fraction of lactate as a result of the process of ensiling. Besides, the fat content of grass silage is lower and less unsaturated than that of fresh grass. Consequently, CH<sub>4</sub> losses in animals fed grass silage are likely to be higher than in animals fed fresh grass. Unlike with fresh grass, no research results are available of a range of grass silage qualities in dairy cows fed with grass silage as the main component (> 90%) of their diet and direct comparisons between fresh grass and grass silage are also lacking. These uncertainties make it difficult to derive firm conclusions on grass silage as compared to fresh grass. But, in agreement with the conclusion of Veen (2000), because of the relatively high cell wall digestibility in Dutch grass silages (table 1), scope for improvement here is considered to be low. Besides, year round indoor feeding is increasing, and the contribution of fresh grass to dairy diets is expected to decrease in the coming years.

### 5.1.3 Maize silage

Because of its high starch content, maize silage is expected to result in lower CH<sub>4</sub> losses than grass silage, despite its low cell wall digestibility (table 2). Replacing half of the 60% of grass silage in the DM of a control ration with maize silage fed to mid-lactation dairy cattle in the Wageningen respiration chambers reduced methane production (though not significantly) from 6.0 to 5.8% of GEI (13.6 and 12.1 g CH<sub>4</sub>/kg milk respectively) (Van Laar and Van Straalen, 2004). Reduced N fertilisation of maize may compromise dry matter yield, but has no effect on the grain:leaf ratio and feeding value (H. van Schooten, pers. comm). Recent data from maize variety test trials (J. Groten, 2006, unpublished data) indicate that the progress in improvement of starch content as well as cell wall digestibility is still continuing. There are indications that considerable differences exist in *in situ* starch degradation characteristics between maize varieties. Some new maize varieties with improved grain to leaf ratios and cell wall digestibility show a net energy content which is more than 0.7 MJ/kg DM higher than current varieties. Further optimization of the stage of maturity at harvest could also be an option to improve the yield and quality of maize silage. In the experiments of Groten, maximum yields of dry matter, NEL and starch were achieved between 35 and 40% DM in the whole crop which is higher than the current recommendation to harvest between 30 and 35% DM (Van Dijk et al. 2006). Improved yields enable a larger proportion of maize silage in the diet of dairy cows. Harvesting at a later stage of maturity also improves starch content (grain to leaf ratio) (Van Dijk et al. 2006). In general, the rumen degradability of starch in maize silage is reduced with increasing stage of maturity. Harvesting at a later stage of maturity may result in a shift of starch digestion from the rumen to the intestine and thereby contribute to a reduction of CH<sub>4</sub> losses. A recent study on the effect of maize varieties with either a high starch content or a high cell wall degradability showed that cows fed maize silage from a high starch variety produced more milk, protein and FPCM than cows fed silage from a variety with a high cell wall digestibility (Zom, 2006). However, the differences in total starch content between the varieties were quite small, but the proportion of *in situ* by-pass starch was much higher (54 vs 32%) in the high starch variety, which may have caused a shift in the site of starch digestion. So there may be some potential to improve starch degradation characteristics by means of plant breeding. However, improved, rapid and low cost methods to estimate *in situ* starch degradation characteristics are needed to identify selection lines with improved starch degradation characteristics.

### 5.1.4 Whole cereal plant silage

Until recently, it was hypothesized that autumn sown Whole Cereal Plant silage crops (WPS) were more efficient in N use because they could catch mineral N from the soil and thereby prevent losses due to leaching. Besides WPS could yield starch and consequently reduce CH<sub>4</sub> losses. However, recent work of Verloop et al. (2006) shows that growing WPS leads to higher leaching than grass or maize which is attributed to its poor growth in the period that it should act as a catch crop. Data from BLGG show that the number of WPS clamps sampled for feed analysis remains stable, suggesting that there is no increase in the use of WPS in the Netherlands.

### 5.1.5 Forage legumes

Research from New Zealand (Ramirez-Restrepo & Barry, 2005) suggests that feeding forage legumes like lucerne or red clover also tends to decrease CH<sub>4</sub> losses (g.kg<sup>-1</sup> DMI) compared to grass. This reduction can be further enhanced by legumes that contain condensed tannins such as sulla (*Hedysarum coronarium*) as will be discussed in more detail in paragraph 5.5.1.

### 5.1.6 In conclusion

Increasing the digestibility of cell walls in forages has been suggested as a means to lower CH<sub>4</sub> losses, but in fresh grass and grass silage the scope of this approach appears to be rather limited. There is evidence that fresh grass results in lower CH<sub>4</sub> losses than grass silage, but no direct comparisons exist between fresh grass and grass silage. Besides, unlike with fresh grass no research results are available of a range of grass silage qualities. Legislation aiming at a reduction of the use of N fertiliser will probably result in forages harvested at a slightly higher maturity which would also not be in favour of reducing CH<sub>4</sub> losses.

Besides, the contribution of fresh grass to dairy diets will likely decrease in the coming years. In view of the new manure legislation in The Netherlands (EU directive on ground water quality), it is expected that the area used to grow maize for silage will become smaller in the coming years, also limiting the possibilities to improve the digestibility of dairy diets by improving digestibility of stem and leaves of maize. Harvesting maize silage at a more mature stage improves the yields and quality of maize silage as a result from a higher grain to leaf ratio (starch to cell wall ratio) and reduced rumen degradability of starch. Maize breeders should put their efforts on improvement of the proportion of by-pass starch. However, this is hampered by a lack of easy, rapid and low cost methods to assess *in vivo* starch degradation characteristics.

It is worthwhile to mention the statement of Veen that, if international trading of emission rights would be allowed there is scope for the Netherlands to give a significant contribution to improvement of the digestibility of forages in Eastern-Europe. Export of knowledge and seeds could be a useful tool to achieve this.

## 5.2 Concentrates

In high producing dairy cows their high requirements often exceeds their capacity to ingest sufficient nutrients from forages alone. As a consequence forages have to be supplemented with concentrates. Compared to forages, concentrates are usually lower in cell wall components. Due to the presence of non-structural carbohydrates (starch and sugars), concentrates normally ferment faster than forage, giving rise to elevated levels of propionic acid. Veen (2000), quoting Beever (1993), suggest that CH<sub>4</sub> production can be lowered by almost 40% (from 272 to 170 g/day) when a forage rich diet is replaced by a concentrate rich diet.

### 5.2.1 The proportion of concentrates

The proportion of concentrates in dairy diets is often included as an explanatory factor in empirical models of CH<sub>4</sub> production (Holter & Young, 1992; Yan et al., 2000). Increasing the dietary proportion of concentrates usually reduces CH<sub>4</sub> losses. This effect appeared independent of the genetic merit (Ferris et al., 1999). The CH<sub>4</sub> reduction is well in line with the observations of Bannink et al. (1997b) that concentrate rich diets showed lower and higher coefficients of conversion of substrate into acetate and propionate respectively.

Increasing the proportion of concentrates is limited by a required minimum level of physical structure in the diet (prevention of (sub-)clinical acidosis) and the balance between energy intake and requirements (prevention of excessive overfeeding) in low producing animals (dry and late lactation cows, young stock). Feeding large amounts of concentrates is sometimes associated with a higher risk for lameness (Manson & Leaver, 1988). High concentrate intake could also result in a high BSC (fat cows) at calving, which is associated with a higher risk for metabolic disorders, and reduced fertility (Kadokawa & Martin, 2006).

Restricting feed intake could be an option to prevent overfeeding. Nowadays, however, it is very uncommon to restrict the access to forage. Restricting feed intake may have a negative impact on animal welfare because of unrest caused by insatiated cows trying to get access to the feeding area or cows that are being bullied at the bunk. Therefore, new feeding systems need to be developed.

### 5.2.2 Carbohydrates in concentrates

Concentrates and concentrate ingredients are quite variable with regard to their content of structural (cellulose, hemicellulose) and non-structural (starch, sugars) carbohydrates. The degradative behaviour of both groups of carbohydrates also varies widely, notably the rate of degradation of starch. Consequently, VFA profile and CH<sub>4</sub> loss vary accordingly. In beef cattle it was shown (Johnson & Johnson, 1995), that digested cell walls normally lead to higher losses than non cell wall components, and that within non cell wall components soluble sugars are more methanogenic than starch.

The effect on CH<sub>4</sub> loss of carbohydrates in concentrates fed to lactating dairy cows was studied by Hindrichsen et al. (2005). The cows weighed 595 kg and were fed diets with a 1:1 forage to concentrates ratio, the forage consisting of maize silage (0.22), grass silage (0.45) and hay (0.33). The animals consumed between 14.2 and 17.3 kg DM d<sup>-1</sup> and produced on average 21 kg of milk. Enteric CH<sub>4</sub> emissions ranged between 351 and 429 g d<sup>-1</sup>, or between 18.3 and 22.1 g.kg<sup>-1</sup> milk, well in line with the observations presented in figure 6. For a total of 35 measurements the following equation was derived:

$$\text{CH}_4 \text{ (g d}^{-1}\text{)} = 84 + 47 \cdot \text{Cellulose (kg d}^{-1}\text{)} + 32 \cdot \text{Starch (kg d}^{-1}\text{)} + 62 \cdot \text{Sugars (kg d}^{-1}\text{)} \quad (R^2=0.794)$$

This equation could be slightly improved by replacing ingested nutrients by digested nutrients:

$$\text{CH}_4 \text{ (g d}^{-1}\text{)} = 91 + 50 \cdot \text{Cellulose (kg d}^{-1}\text{)} + 40 \cdot \text{Hemicellulose (kg d}^{-1}\text{)} + 24 \cdot \text{Starch (kg d}^{-1}\text{)} + 67 \cdot \text{Sugars (kg d}^{-1}\text{)} \quad (R^2=0.843)$$

All carbohydrate fractions yielded CH<sub>4</sub>, but the highest contribution to CH<sub>4</sub> losses came from sugars, of which variation in the diets predominantly resulted from the inclusion of *Jerusalem artichokes* and molasses. The low contribution of starch results from a propionate dominated VFA profile, depending on type of starch source and level of DMI, causing a shift towards post-ruminal digestion of starch. The highest (250 g kg<sup>-1</sup> dOM) and the lowest (50 g kg<sup>-1</sup> dOM) inclusion of starch in the total diet differed in CH<sub>4</sub> losses calculated from the regression equations by about 10%. Combining this with a reduced sugar content could decrease the CH<sub>4</sub> losses to 12.5%. Losses of CH<sub>4</sub> per kg dOM showed indeed a difference of 12.5% between the highest (56 g kg<sup>-1</sup> dOM at dOM=0.684) and the lowest (49 g kg<sup>-1</sup> dOM at dOM=0.744). It remains to be seen if such differences can also be obtained under more practical feeding situations and if they are sustainable. However, the above coefficient values for the different types of carbohydrate are in line with the CH<sub>4</sub> losses that are calculated from VFA profiles estimated by Bannink et al. (2006) from *in vivo* data from lactating cows only.

An additional question is whether differences exist between starch sources depending on degradability and feed processing, and between rapidly fermentable non-starch carbohydrates such as sugar-rich or pectin-rich feedstuffs.

It was of interest to know that CH<sub>4</sub> emission from slurry varied between 16 and 22% of the total system CH<sub>4</sub> emission and, contrary to expectation, a higher faecal excretion of fibre did not increase CH<sub>4</sub> emission from the slurry.

In feedlot cattle fed with barley or corn based diets in the finishing phase (Beauchemin & McGinn, 2005), CH<sub>4</sub> losses amounted to 2.8 and 4.0% of GEI for corn and barley respectively. The low proportion of GEI lost as CH<sub>4</sub> was probably caused by a propionate type of rumen fermentation, the very low figure for the corn based diet must have resulted from a larger proportion of starch in corn escaping digestion in the rumen.

### 5.2.3 In conclusion

Feeding more concentrates per cow, especially those with a higher amount of (rumen resistant) starch and less sugars has a very positive effect on the reduction of CH<sub>4</sub> losses.

## 5.3 The addition of fats and oils

Adding oils to dairy diets has also been recommended as a way to reduce CH<sub>4</sub> losses. According to Veen (2000), possibilities to include more fat in dairy diets is limited, because feeding fat of animal origin is forbidden and many vegetable fats often do stimulate milk production, but have a negative influence on fat- and protein content of the milk. According to Veen (2000), attention should be paid to the use of fish oils, because there are indications that they might reduce CH<sub>4</sub> emission without showing a negative effect on cell wall digestibility in the rumen.

*In vitro* research, using the RUSITEC system showed that fatty acids (FA) with medium chain length, notably lauric (C<sub>12:0</sub>) and myristic (C<sub>14:0</sub>) acid, when added at a level of 53 g kg<sup>-1</sup> DM, severely inhibited CH<sub>4</sub> production (Dohme et al., 2000). Further research (Dohme et al., 2001), showed that, when added at a level of 50 g kg<sup>-1</sup> DM, caprylic (C<sub>8:0</sub>), capric (C<sub>10:0</sub>), palmitic (C<sub>16:0</sub>), and stearic (C<sub>18:0</sub>) did not inhibit CH<sub>4</sub> production *in vitro*, but linoleic acid (C<sub>18:2</sub>) did.



The subject of fat inclusion in diets of dairy cows *in vivo* was reviewed by Giger-Reverdin et al (2003). From a data base of 37 experiments, mainly obtained in short term metabolic trials, they concluded that CH<sub>4</sub> losses in dairy cows could be predicted with an equation in which DMI and the FA content were included:

$$\text{CH}_4 \text{ (g kg}^{-1} \text{ DMI)} = 47.2 - 0.0217 \cdot \text{DMI}^2 - 0.735\% \text{FA/kg DMI} \text{ (R}^2 = 0.76; \text{rsd} = 2.62/\text{kg DMI})$$

This equation could be further improved by separating FA in saturated FA, monoene FA, C<sub>18:2</sub>, C<sub>18:3</sub> and FA with a chain length of 20 C ( $\Sigma \text{C}_{\geq 20}$ ) or more.

This yielded:

$$\text{CH}_4 \text{ (g kg}^{-1} \text{ DMI)} = 45.0 - 0.018 \cdot \text{DMI}^2 - 1.84 \cdot \text{C}_{18:2} - 84.2 \cdot \Sigma \text{C}_{\geq 20} \text{ (R}^2 = 0.81; \text{rsd} = 2.37/\text{kg DMI})$$

More recent research has studied the effects of sunflower oil (rich in linoleic acid, C<sub>18:2</sub>) in beef cattle in Canada (McGinn et al., 2004), refined coconut oil and copra meal (both rich in lauric acid, C<sub>12:0</sub>) in beef heifers in Ireland (Jordan et al., 2006), and canola oil (rich in oleic, C<sub>18:1</sub> and linoleic acid, C<sub>18:2</sub>) in growing beef cattle in Canada (Beauchemin & McGinn, 2006). Recent experiments with dairy cattle include mixtures of whole cottonseed (rich in linoleic acid, C<sub>18:2</sub>) and canola meal in the USA (Johnson et al., 2002), medium chain fatty acids in dairy cows in Switzerland (Dohme et al., 2004), and mixtures of sunflower oil and fish oil (rich in arachidonic oil, C<sub>20:4</sub>) to dairy cows in New Zealand (Woodward et al., 2006).

In beef cattle, the addition of sunflower oil (400 g.d<sup>-1</sup> or 5% of DMI) decreased CH<sub>4</sub> emissions by 22% with no negative effect on DM intake, but reductions in DM and NDF digestibility were 9% (from 62.0 to 58.2) and 23% (from 44.3 to 34.1), respectively (McGinn et al., 2004). The addition of coconut oil (250 g.d<sup>-1</sup>) to a 50/50 grass silage to concentrate ratio diet of beef cattle in Ireland (Jordan et al., 2006), either as refined oil or as copra meal decreased CH<sub>4</sub> loss by between 15 and 20% when expressed in L.d<sup>-1</sup>, kg<sup>-1</sup>.DMI or as % of GEI, without showing negative effects on DMI or digestibilities of DM or NDF. Canola oil (6% in DM) in the diet of Angus heifers fed diets of barley silage (75%) and concentrates (80% barley grain) reduced CH<sub>4</sub> emissions by 32%, primarily due to a decreased DMI together with a lowered total tract digestibility of DM and fibre (Beauchemin & McGinn, 2006).

In the long term study of Johnson et al. (2002), Holstein cows (n=36) were fed three diets with 51.4% forage (a mixture of 35% lucerne hay and 65% lucerne silage) and 48.6 % concentrates (66% of which was barley and corn), that contained 2.3; 4.0 and 5.6 % fat (a 1:2 mixture of canola and whole cottonseed) from the day of calving until 305 days in milk (DIM). Methane was measured using the SF<sub>6</sub> tracer technique. Fat addition increased DMI by 7.9 and 6.7 % for medium and high fat addition. Milk yield (33.9 kg FCM d<sup>-1</sup> with the control diet) was increased by 17% with both the fat rich diets. The control diet caused an extremely low loss of only 4.7% of the GEI to be lost in CH<sub>4</sub> and the addition of fat did not show a significant reduction (4.2% at the medium and 4.9 at the high fat addition).

In the study of Dohme et al. (2004), lauric (C<sub>12:0</sub>), myristic (C<sub>14:0</sub>) and stearic (C<sub>18:0</sub>) acid were added at a level of 40 g kg<sup>-1</sup> DM to the diet of 18 Brown Swiss dairy cows fed a 3:2 forage (mixture of 51% grass silage, 32% maize silage and 17% hay) to concentrates (66% barley) ratio, producing between 25 and 30 kg of milk d<sup>-1</sup>.

Experimental periods were 25 days, including 10 d of adaptation. Compared to stearic acid, the addition of lauric acid decreased feed intake by 18%, reduced NDF digestion by 7% and reduced CH<sub>4</sub> loss (g CH<sub>4</sub> kg<sup>-1</sup> milk) by 16%. Myristic acid showed little difference with stearic acid, except a reduction in CH<sub>4</sub> of almost 8%.

Woodward et al. (2006) added mixtures of sunflower and fish oil (500 g d<sup>-1</sup>) to the diet of dairy cows (n=32) fed pasture based diets. In short term trials (14 days) oils had no effect on DMI or milk yield, but reduced CH<sub>4</sub> by 27% (13.5 vs 18.5 g CH<sub>4</sub> kg<sup>-1</sup> DM). However, in a long term trial (12 weeks) the addition of oil (300 g linseed and fish oil) to the diet of grazing cows (n=20) had no effect on CH<sub>4</sub> emissions (21.7 vs. 23.0 g CH<sub>4</sub> kg<sup>-1</sup> DM).

Currently, oil prices are rising because of the increasing demands for oil by booming economies in Asia and political instability of oil producing regions. High oil prices are a driving force behind the increased use of bio-fuels which puts pressure on the markets of vegetable oils. Therefore, it is expected that prices of vegetable oil will rise and remain high in the future. High oil prices reduce the chances of vegetable fats and oils as a cost-effective measure to reduce CH<sub>4</sub> losses. Therefore, introduction of new home grown oil-rich forage crops such as high-oil maize could be interesting. The fatty acid concentration of the diet can be increased by replacing conventional maize silage or grain by maize silage or grain from high oil maize varieties (LaCount et al., 1995; Weiss & Wyatt, 2000; Whitlock et al., 2003). Fatty acid content of high oil corn silage is 2 percent units higher than in maize silage of conventional hybrids (LaCount et al., 1995; Weiss & Wyatt, 2000). The grain of high oil maize is typically reported to contain 7 to 8% ether extract, which is twice as much as in conventional maize grains. Also replacing purchased concentrates by home-grown concentrates such as CCM or ground maize ear silage from high oil

varieties could be an option. A positive side effect of the production of home grown concentrates is a reduced usage of fossil fuel for the production and transport of purchased concentrates. High oil maize varieties can be easily produced by means of the TopCross system in which (artificial) male sterile plants of a conventional variety are pollinated by a high oil variety. Research with feeding high-oil maize silage resulted generally in no or a small increase in milk production (LaCount et al., 1995; Weiss & Wyatt, 2000). However, derogation from the EU directive on ground water quality, limits the proportion of farm land that can be used for growing maize. This means that the proportion of maize silage is limited to approximately 50% of the total forage.

### 5.3.1 *In conclusion*

Results of adding fats to diets of cattle on CH<sub>4</sub> emission are variable and seem to be influenced by the type of FA (chain length, degree of unsaturation), the type of animal (beef vs. dairy cattle), the type of diet (forage vs. concentrate rich), and the length of the experimental period. Next to reduced CH<sub>4</sub> losses, reductions in DMI and cell wall digestibility have often been observed. In long term trials adaptations of the microflora seem to occur. High oil prices may make the inclusion of vegetable oils in dairy diets less competitive.

## 5.4 Feed additives

Improvement of the microbial efficiency in the rumen has received attention for a long time already, because this positively influences the N utilisation. Veen (2000) is of the opinion that developments in this direction deserve to be stimulated further, despite the fact that the use of certain feed additives, like anti-microbials, has a low societal acceptability or is forbidden already. Nevertheless, a further development of knowledge in this area should be stimulated.

Several bioactive compounds among which were essential oils, ionophores, saponin containing plant extracts, surfactants and tannins were investigated *in vitro* for their protozoa reducing activity (Hristov et al., 2003). Some of them inhibited protozoa, but this was often accompanied with a decrease in polysaccharide degrading activity.

Ionophores, notably monensin, have been suggested as depressing agents for CH<sub>4</sub> production in ruminants and were discussed by Moss et al. (2000). It appeared from long term trials that their effect was not persistent. Besides, antimicrobials including monensin, are banned in the EU.

A wide range of plant materials that could modify rumen fermentation has recently been investigated in 'RUMEN-UP', a EU sponsored shared cost action (Wallace, 2004), followed by 'REPLACE' with a focus at the use of plant compounds to improve forage utilization by ruminants and to replace antibiotics in the diet of monogastrics. The latter seems to be the predominant goal of REPLACE.

Momentarily, several methods of manipulating rumen methanogenesis are being explored in several countries, ranging from feeding of plant extracts, myristic acid, fats, to the long-term feeding of monensin, introducing acetogens, and effects of grass quality and grain processing (Kebreab, pers.comm.).

### 5.4.1 *Organic acids*

It has also been suggested that the addition of organic acids, the intermediates of carbohydrate degradation in the rumen, would stimulate the production of propionic acid in the rumen and could reduce CH<sub>4</sub> losses (Castillo et al., 2004), by acting as a H<sub>2</sub> sink. Newbold et al. (2005) tested 15 potential precursors of propionate, including pyruvate, lactate, fumarate, acrylate, malate and citrate, in short-term batch cultures. Sodium acrylate and sodium fumarate produced the most consistent effect decreasing CH<sub>4</sub> production by between 8 and 17%. Free acids rather than salts were more effective in reducing CH<sub>4</sub>, but also decrease pH with possible negative effects on fibre degradation. In longer term (21 d) *in vitro* incubations, fumarate addition decreased CH<sub>4</sub> production by 28% whilst maintaining DM degradation, whereas malate was not effective. Adding malate to an *in vitro* rumen fermentation system in which maize, barley, wheat or sorghum were used as a substrate, only marginally reduced CH<sub>4</sub> production (Carro & Ranilla, 2003). The addition of fumaric acid to a continuous rumen culture with ryegrass pasture as a substrate, depressed CH<sub>4</sub> production with a concomitant increase of propionic acid (Kolver et al., 2004). However, the addition of fumaric acid to diets of Holstein steers (80 g d<sup>-1</sup>) or Angus heifers (175 g d<sup>-1</sup>) in Canada (McGinn et al., 2004; Beauchemin & McGinn, 2006), had no measurable effect on CH<sub>4</sub> emissions.

#### 5.4.2 Essential oils

Essential oils are the volatile components responsible for the characteristic aroma of spices. They are considered as plant secondary metabolites and may have antimicrobial properties. The application of a blend of essential oil compounds (major components thymol, guajacol, limonene) to the rumen of mature sheep (110 mg d<sup>-1</sup>) were small (Newbold et al., 2004) and seemed restricted to a reduced deamination. The addition of a similar extract of essential oils (1 g d<sup>-1</sup>) to diets of beef cattle in Canada had no measurable effect on CH<sub>4</sub> emissions (Beauchemin & McGinn, 2006).

Other potential additives that could have effects are yeast and enzymes, but no effects on CH<sub>4</sub> losses have been observed in beef cattle (McGinn et al., 2004).

#### 5.4.3 In conclusion

A number of feed additives have shown potential as inhibitors of CH<sub>4</sub> in *in vitro* experiments. However, in the scarcely available long term *in vivo* experiments they were often without any effect, probably because of adaptation of the rumen microbial system.

### 5.5 Plant secondary metabolites

To protect themselves against microbial and insect attack, plants produce a variety of secondary compounds. Some of them are also toxic to animals, but others are not. Many of such compounds have been used as whole plants or plant extracts for food or medical application in man. Research on the effect of plant secondary metabolites, notably condensed tannins (Ramirez-Restrepo & Barry, 2005), essential oils and saponins (Wallace, 2004), is receiving much attention these days, primarily with the aim that secondary plant metabolites can possibly replace antimicrobials. As a side effect, in some instances inhibiting effects on CH<sub>4</sub> have been observed, most likely mediated through an effect on rumen protozoa.

#### 5.5.1 Condensed tannins

An interesting development could be the introduction of new forage varieties with elevated levels of condensed tannins, such as clover and other legumes like trefoil, vetch, sulla, and cichory. In New Zealand sheep, housed indoors and fed with different forages (lucerne, sulla, red clover, cichory and lotus), CH<sub>4</sub> losses (g CH<sub>4</sub>/kg DMI) were reduced by between 20 and 55% as compared to animals pastured on ryegrass/white clover mixtures (Ramirez-Restrepo & Barry, 2005).

In goats, fed with the condensed tannin containing forage sericea lespedeza (*Lespedeza cuneata*), Puchala et al. (2005) observed in Oklahoma (USA) a reduction in CH<sub>4</sub> loss of over 30% compared with goats fed with a mixture of crabgrass (*Digitaria ischaemum*) and tall fescue (*Festuca arundinacea*).

Similar observations have been made in grazing dairy cows in New Zealand grazing sulla (*Hedysarum coronarium*) or lotus (*Lotus corniculatus*) in stead of perennial ryegrass (Ramirez-Restrepo & Barry, 2005). These authors consider cichory (*Chichorium intybus*) also a promising forage to reduce CH<sub>4</sub> losses in ruminants. In New Zealand chicory is used as a forage under grazing and its agronomic and nutritive value was reviewed by Barry (1998). Climatic, agronomic and nutritional limitations are thought to limit their practical use in The Netherlands, but it is of interest to know that both lotus and chicory are being used as forages under grazing in Uruguay (P. Chilibroste, personal communication).

The potential to incorporate forage crops with elevated levels of condensed tannins in dairy farm systems is low. Red clover, vetch and sulla are not winter hardy crops and cichory is an annual plant, therefore they need to be reseeded each year. A possible reduction of CH<sub>4</sub> is at least partly counteracted by an increased loss of CO<sub>2</sub> from the soil and tractor fuel used for tillage. Growing birdsfoot trefoil (Lotus) seems to have a better prospective. However, there is little knowledge about the agronomy, conservation and feeding value of trefoil, which is a barrier for a widespread use.

### 5.5.2 Saponins

Saponins are naturally occurring surface-active glycosides, occurring in many plant species, wild plants as well as cultivated crops. They usually consist of a sugar moiety linked to a hydrophobic compound, either triterpenoid or steroid in nature (Francis et al., 2002). Saponin containing plants and their extracts have been shown to suppress the bacteriolytic activity of rumen ciliate protozoa (Moss et al, 2000). Saponins are considered to have detrimental effects on protozoa through their binding with sterols present on the protozoal surface (Francis et al., 2002). Because of their anti protozoal activity, saponins might have potential to reduce CH<sub>4</sub>. Some of them have shown positive effects *in vitro* (Pen et al., 2006), but it has also been shown that microbial adaptation to saponins may occur (Wallace, 2004). No long term *in vivo* studies with cattle have been reported.

### 5.5.3 In conclusion

A number of plant secondary metabolites have shown some potential as inhibitors of CH<sub>4</sub> in *in vitro* experiments. This seems notably the case with condensed tannins that reduced CH<sub>4</sub> losses both in *in vitro* and *in vivo* experiments.

## 5.6 Microbial ecosystem

Research on changes in the profile of the microbial ecosystem in the rumen is still in its infancy. The necessary molecular and biotechnological techniques may meet societal resistance, but that should not be a reason for hampering scientific research. Recent research (Vlaeminck et al., 2006) suggests that the microbial ecosystem in the rumen is reflected in the fatty acid profile of milk fat. This seems particularly true for odd- and branched-chain fatty acids (OBCFA). These acids are synthesized by the rumen bacteria and their excretion in milk reflects the microbes and microbial activity. The OBCFA have been used to predict the duodenal flow of bacterial crude protein and the rumen fermentation pattern. Although additional studies are needed to elucidate and verify the relationship between milk OBCFA and CH<sub>4</sub> production, if proven valid, this would yield an important non-invasive tool to study changes in CH<sub>4</sub> losses.

## 5.7 Alternating strategies

The effects of including fats, plant secondary metabolites and additives on CH<sub>4</sub> excretion seem to be non-persistent. This problem could possibly be solved by alternating different feeding strategies. For example: in early lactation feeding high (by-pass) starch and concentrate diets, followed by feeding high fat diets in mid-lactation, and feeding forages high in tannins in late lactation. A possible side effect of such strategy might be that cows are fed closer to their nutrient requirements. The effect of such alternating feeding strategies should be explored.

## 6 Side-effects of attempt to reduce CH<sub>4</sub>

When trying to mitigate losses of GHG by nutrition one should be aware of possible side effects, for instance on product quality, on animal welfare and health, and on the release of other GHG. Also Veen (2000) emphasized the need address animal welfare and health besides positive effects on milk yield and CH<sub>4</sub> loss. Detrimental effects on cow performance specifically mentioned were the risk of (subclinical) rumen acidosis with feeding more concentrates, the positive effect of grazing instead of stall-feeding on cow health. Some further examples of side effects of attempts to reduce CH<sub>4</sub> are discussed below.

### 6.1 Dietary manipulation

As indicated before, dietary changes are a promising means to reduce CH<sub>4</sub> losses. Such changes may well affect the composition of the products. This section focuses on the impact of dietary means to reduce CH<sub>4</sub> losses on milk composition.

#### 6.1.1 Milk lactose

Dietary effects on milk lactose are usually very small (Jenkins and McGuire, 2006). Milk is isotonic with respect to blood. Lactose contributes some 60% of milk osmolality; K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> contribute to most of the other 40% (Peaker, 1977). In view of the insensitivity of milk lactose content to dietary changes, reduction of CH<sub>4</sub> losses by dietary means is not expected to affect milk lactose to a great extent.

#### 6.1.2 Milk protein

Milk crude protein can be distinguished into various fractions, viz. casein protein (some 80%), whey protein (15%) and non-protein-N (such as urea; 5%). All the casein proteins, as well as the whey proteins  $\alpha$ -lactalbumin, lactoferrin and  $\beta$ -lactoglobulin, are synthesized in the mammary gland, whereas other whey proteins including immunoglobulins and serum albumen are derived from blood (DePeters & Cant, 1992). Nutrition has very little effect on the relative appearance of various protein fractions in the milk and this will not be considered further. Total milk protein content is more responsive to dietary changes than is milk lactose. The change in milk protein due to dietary changes is restricted to a 0.5 percentage unit range (Jenkins & McGuire, 2006).

##### 6.1.2.1 Protein level and source

On a weight basis, CH<sub>4</sub> production from fermented protein is lower than that from fermented carbohydrates (Bannink et al., 2005). Increasing the level of dietary crude protein has no consistent or only a small positive effect on milk protein concentration, provided that cows are not in a state of severe protein undernutrition (Beever et al., 2001), which in The Netherlands rarely occurs. Thus CH<sub>4</sub> reduction by increasing protein concentration is expected to give no, or only minor positive, responses in milk protein. However, as explained in section 6.4, it results in an increased excretion of N in manure that easily leads to an increased loss of N<sub>2</sub>O.

##### 6.1.2.2 Energy level and source

Milk protein concentration is positively correlated with diet metabolizable energy (ME) concentration (DePeters & Cant, 1992), except when this energy is provided by lipids (for discussion of lipids, see next paragraph). Such an increase in ME level in experiments is often caused by a changed roughage:concentrate ratio. Usually, higher concentrate levels and hence higher ME levels correspond with increased proportions of propionic acid in the rumen, and thus decreased levels of CH<sub>4</sub> production per unit fermented carbohydrate. From various studies it appears that higher propionic acid levels induced by starch rich diets or by higher DM intake levels coincide with higher insulin levels in plasma, leading to signals in the body of the cow to produce more milk protein (Jenkins & McGuire, 2006). Thus in general, increasing starch content of the diet to reduce CH<sub>4</sub> may increase milk protein and milk protein production.

##### 6.1.2.3 Fat level and source

Feeding additional fat often causes a small reduction in milk protein content (Jenkins & McGuire, 2006). Fat supplements may reduce blood flow through the mammary gland causing reduced extraction of blood amino acids (Cant et al., 1993). However, since additional feed fat generally increases milk production, total milk protein

production may not be decreased. Unsaturated fat supplements appear to have a higher milk protein reducing effect than saturated fat supplements. In the experiment of Dohme et al. (2004) the effects of the saturated FA lauric, myristic and stearic acid did not show significant differences in milk protein concentration. Thus, the use of unsaturated FA to decrease CH<sub>4</sub> may reduce milk protein content, whilst lauric and myristic acid will have no or a less pronounced effect on milk protein content.

#### *6.1.2.4 Additives and plant secondary metabolites*

Knowledge of the effects of additives on milk protein concentration is limited. Usually, yeast supplementation does not affect milk protein concentration (Erasmus et al., 2005). Ionophores such as monensin are not allowed to be used for dairy cattle in Europe. In other countries, experiments with monensin typically indicated no changes in milk protein content to occur (Ipharraguerre & Clark, 2003). Condensed tannins may increase protein flow to the duodenum and especially in situations where dietary crude protein level is low and is limiting an efficient production of microbial protein, condensed tannins may lead to a slightly increased milk protein content (Bhatta et al., 2000; Woodward et al., 2000). A very limited number of studies also showed no differences in milk protein concentration upon supplementing cows with malic acid (Sniffen et al., 2006).

#### *6.1.3 Milk fat*

The most sensitive component of milk to dietary manipulation is its fat content (Jenkins and McGuire, 2006). Milk fat comprises a range of saturated and unsaturated FA that differ also in chain length. Fatty acids up to a chain length of 16 carbon atoms (C<sub>16</sub>) are synthesized de novo in the gland from precursors including acetic acid and β-hydroxy butyric acid. Typically about half of the C<sub>16</sub>, and all C<sub>18</sub> and longer chain FA, are derived from circulating plasma lipids. The extensive biohydrogenation of FA in the rumen, in which hydrogen is used and which therefore will potentially reduce CH<sub>4</sub> production in the rumen, prevents high proportions of unsaturated FA to occur in the milk. Oleic acid (C<sub>18:1</sub>) is the largest contributor to milk unsaturated FA, and most of the oleic acid is formed from stearic acid (C<sub>18:0</sub>) by desaturase activity within the mammary gland. Trans FA in milk occur largely because of the isomerization processes in the rumen. Some of these trans FA, notably trans-10 FA isomers, reduce de novo synthesis of FA in the gland, causing milk fat depression and a higher proportion of milk FA with a chain length of 18 carbon atoms or higher (Bauman et al., 2006).

There are many effects of individual FA on human health. As a general rule, the smaller-sized saturated FA (up to 16 carbon atoms) raise 'bad' cholesterol levels (LDL-cholesterol) or have a negative impact on the ratio between LDL-cholesterol and total cholesterol, with associated increases in cardiovascular disease risks (Givens & Shingfield, 2004). Lauric, myristic and palmitic acid have a particularly strong effect on raising LDL cholesterol, whereas stearic acid is neutral. Unsaturated FA, in particular omega-3 and omega-6 FA, may reduce LDL-cholesterol levels. In addition, butyric acid and particularly conjugated linoleic acids (CLA; a mixture of geometric and positional isomers of C<sub>18:2</sub> that contain a conjugated double bond in which cis-9, trans-11 C<sub>18:2</sub> is the most common form) exhibit anti-carcinogenic properties (Lock & Bauman, 2004).

##### *6.1.3.1 Energy level and source*

Milk fat content is usually negatively correlated with diet energy content (Jenkins & McGuire, 2006), again with the exception of energy provided by lipids. An increase in dietary energy content due to a shift in roughage:concentrate ratio will increase the production of propionic acid and the production of trans-C<sub>18:1</sub> fatty acids in the rumen. Both are associated with reduced de novo synthesis of shorter chain fatty acids (smaller than C<sub>16</sub>) in the mammary gland, and with increased proportions of longer chain fatty acids (C<sub>18</sub> and higher) in milk (Chilliard et al., 2000). However, such an effect of concentrate proportion in the diet is more pronounced at low roughage proportions. Cows fed grass silage based diets typically have lower CLA milk contents than cows fed fresh pasture (Elgersma et al., 2006). A reduction in the use of N fertilizer will probably result in forage harvested at a more mature phase. More mature forage will decrease the CLA and C<sub>18:3</sub> content of milk fat but increase the C<sub>16:0</sub> content. Replacing grass silage with maize silage may increase proportions of saturated fatty acids and reduce the proportion of C<sub>18:3</sub> in milk fat, probably related to the lower C<sub>18:3</sub> and higher C<sub>18:1</sub> contents of maize silage compared with grass silage (Dewhurst et al., 2006). Overall, increasing concentrate proportions to reduce CH<sub>4</sub> emissions may reduce milk fatty acid content and increase the proportion of various unsaturated fatty acids. Using more maize silage at the expense of grass silage to reduce CH<sub>4</sub>, however, may increase the proportion of saturated fatty acids in milk.

##### *6.1.3.2 Fat level and source*

The effects of various fat supplements on milk fat content and composition are variable. Including plant oils (e.g., linseed oil, rapeseed oil, sunflower oil) in the diet generally results in a reduction in the proportion of shorter fatty

acids ( $C_{16}$  or less) and a usually small increase in unsaturated fatty acid proportions (Chilliard et al., 2000). Changes in milk fat unsaturated fatty acids are likely directly related to increases in concentration of these fatty acids predominant in plant oil supplements, while rumen metabolism of unsaturated fatty acids in some plant oils may lead to increased CLA levels. For example, since recently, compound feeds are being produced in the Netherlands containing NUTEX (extruded linseed) which increases the milk content of unsaturated and omega-3 fatty acids. Although introduced in the market with the aim to change milk fat composition, inclusion of such special oils in the dairy ration reduces  $CH_4$  production as well. Addition of rumen protected fat sources in the diet gives much larger shifts in the milk fatty acid profile, but obviously protected fat is expected not to have an impact on  $CH_4$  production in the rumen. The use of lauric or myristic acid to reduce  $CH_4$  production has clear adverse effects on milk fat composition, since addition of these fatty acids to the diet also increased their proportions in milk fat (Dohme et al., 2004). Among the most consistent effect on milk fat content is the decrease upon supplementation with omega-3  $C_{20}$  or  $C_{22}$  fatty acids (in fish oil or in marine algae) (Chilliard et al., 2000). Such supplements, rich in omega-3  $C_{20}$  or  $C_{22}$  fatty acids, usually have a pronounced negative effect upon the proportion of saturated fatty acids and a positive effect upon CLA proportion. It should be noted that adverse flavour as a result of this supplementation prevents high inclusion levels in the diet. Thus, the use of unsaturated fatty acids to reduce  $CH_4$  production, in particular omega-3  $C_{20}$  or  $C_{22}$  fatty acids, generally shifts the milk fat profile to a more desired profile, whereas the use of lauric or myristic acid does not give a favourable fatty acid profile shift.

### 6.1.3.3 Additives and plant secondary metabolites

Similar to milk protein, knowledge of the effects of additives on milk fat concentration and profile is limited. Although yeast supplementation may increase fibre degradation and increase VFA production, no effect on milk fat concentration was observed (Erasmus et al., 2005). In countries where ionophore use is permitted for dairy cattle, addition of monensin typically reduced milk fat content (Ipharraguerre and Clark, 2003). The positive effect of ionophores on milk CLA content may well be transient though (Chilliard et al., 2000). Condensed tannins did not influence milk fat content (Bhatta et al., 2000). A limited number of studies showed no differences, or sometimes a small increase, in milk fat content upon supplementing cows with malic acid (Sniffen et al., 2006).

## 6.2 Animal health

In early lactation, feeding more glucogenic nutrients due to more starchy concentrates, would not only reduce the emission of  $CH_4$ , it is also expected to result in a smaller negative energy balance and an improved fertility (Van Kneegsel et al., 2005). Feeding more lipogenic nutrients (including fats and oils) had ambiguous results on energy balance and caused an undesired increase in plasma non-esterified fatty acid (NEFA) and  $\beta$ -hydroxy butyric acid (BHBA), which may be related to increased ketosis and fatty liver syndrome. Positive effects on fertility have also been claimed with the inclusion in the diet of dairy cows in early lactation of rumen protected long chain fatty acids, notably the inclusion of polyunsaturated fatty acids (PUFA; Staples & Thatcher, 2001).

## 6.3 Other green house gases and ammonia

Except  $CH_4$ , other GHG considered important are carbon dioxide ( $CO_2$ ) and nitrous oxide ( $N_2O$ ). In addition gaseous ammonia ( $NH_3$ ) is considered detrimental for the environment. Nutritional interventions should therefore not only take into account the effect on  $CH_4$  losses, but also consider possible effects on the emission of other GHG and  $NH_3$ . It should further be realised that nutrition is not the only cause of GHG emissions. Interventions in farm management practices should therefore be considered in an integrated way. A first such an attempt for dairy farming in The Netherlands was recently published by Schils et al (2005). They divided an intensive dairy farm into 5 components, animal, feed, manure, crop and soil. Interventions were using less mineral fertiliser (1), applying less grazing (2), producing more milk per cow (3) and no grassland renovation (4). They demonstrated that having more milk per cow not only reduced the emission of  $CH_4$ , but also that of  $N_2O$  and  $NH_3$ . Applying less grazing on the other hand increased the emissions of  $CH_4$  and  $NH_3$ , but decreased the emission of  $N_2O$  to the same extent as  $CH_4$  in terms of  $CO_2$  equivalents.

Grazing is considered an important cause of  $N_2O$  emission (Saggart et al., 2004). Hence, when grazing is restricted this might increase the loss of  $CH_4$  from ruminants somewhat, but it would substantially decrease the loss of  $N_2O$ . Because the global warming potential of  $N_2O$  is about 15 times higher than that of  $CH_4$ , this would be an associated advantage. Also Oudendag & Kuikman (2004) concluded that the increased emissions of  $CH_4$  and  $N_2O$  from manure storage and with manure application is more than compensated by the reduction in  $N_2O$  loss

associated with grazing, resulting in a net reduction of loss of CO<sub>2</sub> equivalents. When a reduction of CH<sub>4</sub> losses is associated with a reduced cell wall digestion, one may also expect an elevated hindgut fermentation with a concomitant increased faecal output of cell walls as well as microbial protein. The resulting shift in N excretion from urine to faeces could even further reduce the release of N<sub>2</sub>O. The approaches of budgetting whole farm GHG emissions do not take into account the changes in CH<sub>4</sub> loss from ruminants and the changes in manure characteristics and associated emissions of CH<sub>4</sub> and N<sub>2</sub>O (Oudendag & Kuiman, 2004; Schils et al., 2006). It is not clear what importance needs to be attributed to these changes with respect to the changes in total loss of CO<sub>2</sub> equivalents from dairy farms.

When the reduction in CH<sub>4</sub> losses is brought about by feeding more concentrates or more starch, the reduced CH<sub>4</sub> would be associated by an elevated loss of CO<sub>2</sub>, because of the use of more fossil energy. Based on data on the input of fossil energy of feed ingredients, compiled by Hageman & Mandersloot (1994), Tamminga (1996) calculated that by increasing milk yield from 15 to 40 kg.d<sup>-1</sup>, the contribution of fossil energy would have to increase from 8.4 to 20.8% of the GEI. Based on the regression derived from figure 6 the CH<sub>4</sub> output would be reduced from 20.9 to 10.0 g.CH<sub>4</sub> kg<sup>-1</sup> milk. The reduction in CH<sub>4</sub> release by increasing milk yield due to an increased input of concentrates, would thus be offset by the increased release of CO<sub>2</sub> due to the higher input of fossil fuel. It is recommended that a compilation similar to that of Hageman & Mandersloot (1994) is repeated and made up to date.

#### 6.4 In conclusion

Methane mitigation strategies may have pronounced beneficial or adverse effects on composition of milk. Such effects need to be included in evaluating dietary changes to reduce methane production. As was also concluded by Kebreab et al. (2006b), the entire cycle of GHG formation from feed formulation, animal metabolism, excreta treatment and storage to field application of manure should be studied in an integrated way.



## 7 Practical implementation of dietary measures to reduce CH<sub>4</sub> loss

Dietary measures to reduce the CH<sub>4</sub> loss from dairy herds involve:

- a. Improved forage quality
- b. Larger proportion of concentrate in the diet
- c. More rumen resistant starch
- d. Adding fats and oils
- e. Secondary plant metabolites
- f. Feed additives

The effects of dietary measures on CH<sub>4</sub> emission from cattle are already discussed in a previous section (paragraphs 4.1 till 4.6), and the side-effects in section 6. On a short term, options a, b, c, and d seem to be most promising, because they are immediately available and can be applied instantly. The developments regarding secondary plant metabolites and feed additives are still in a premature state. Therefore, the latter options may become available on the long term. The implementation of dietary measures to reduce CH<sub>4</sub> loss from dairy farms is constrained by the nutritional needs of the different groups of animals within the whole herd. A dairy herd can be divided according to the nutritional needs into different feeding groups according to the stage of lactation (Days in Milk, DIM), level of production and age.

In general, for dairy cows a distinction is made between early lactation cows (<100 DIM), mid lactation cows (100-250 DIM) and late lactation and dry cows (>250 DIM). In addition young stock falls apart in calves (<1 yr of age), (4), and rearing heifers (>1 yr of age), (5). For each feeding group the feasibility of the dietary measures to reduce CH<sub>4</sub> loss on dairy farms will be discussed.

### 7.1 Early lactation cows

Early lactation dairy cows are characterized by a strong increase in milk yield and feed intake capacity. However, the increase of feed intake capacity lags behind the increase in milk energy output causing a negative energy balance (NEB). Therefore, it is common practice to 1) allocate the best quality forages and 2) feed relatively large amounts of concentrates to early lactation and high yielding cows in order to reduce NEB. So, there is little space for further improvement of forage quality and higher levels of concentrate feeding. In addition, feeding larger amounts of concentrates is limited because of the need to maintain sufficient physical structure in the diet.

One strategy could be to increase the proportion of maize silage in the diet. When there is a limited amount of maize silage available (e.g. due to derogation, limiting soil conditions), farmers should consider to (re-)allocate forages among feeding groups such that maize silage is shifted from low-yielding animals to high-yielding animals. A second strategy could be harvesting more mature maize silage to improve the proportion of starch and by-pass starch in maize silage. Re-allocation of forage and harvest more mature maize silage are cost effective measures.

A third strategy is increasing the proportion of by-pass starch in concentrates. This option is in particular interesting for farmers which are not able to grow maize or where growing maize is undesired (e.g. peat soils). In all options to increase starch or bypass starch, next to a reduction of the emission of CH<sub>4</sub> such measure can also be helpful to reduce the NEB of early lactation dairy cows (Van Knegsel et al., 2005).

The addition of fats and oils to diets of early lactation cows seems attractive. Beside a reduction of the emission of CH<sub>4</sub>, this measure may, in specific situations, also be helpful to reduce the NEB of early lactation dairy cows because of the high energy content of fats and oil. A literature survey by Van Knegsel et al. (2007) indicated variable effects of fat addition, however, whereas addition of glucose precursors almost invariably showed positive effects on NEB.

On the short term, there is limited scope for forages with secondary plant metabolites (e.g. tannins). Winter hardy lotus (Birdsfoot trefoil; *Lotus Corniculatus L.*) seems to have the most promising prospects as an alternative forage crop. However, a lot of work needs to be done to improve the agronomy and varieties. The feeding value of lotus is somewhat lower than good quality grass, grass silage and maize silage. Therefore, this (future) option seems less suitable for high yielding cows.

## 7.2 Mid lactation cows

The energy balance of mid lactation dairy cows is slightly above zero, milk yield declines and feed intake capacity is still high. Mid-lactation cows are able to consume sufficient energy from forage supplemented with moderate amounts of concentrate. All dietary measures mentioned above can be applied in mid-lactation dairy cows. However, a side effect of these measures is an increased energy intake. This can be a risk for mid-lactation cows with a relatively low energy requirement. Therefore, these dietary measures should be applied with care because of the risk of over feeding.

## 7.3 Late lactation and dry cows

Overfeeding of late lactation and dry cows should be avoided. High body condition scores in dry cows are associated with metabolic disorders and calving problems.

Therefore, feeding large quantities of high quality forage and concentrates to late lactation and dry cows is no option because it would result in overfeeding. For the same reason, feeding more rumen resistant starch and the addition of fats and oils to the diets of low yielding cows is not an option either. A solution could be to limit the energy intake by a restriction of voluntary feed intake by feeding more or a lower quality forage. However, limiting intake would require an individual feeding system which prevents cows from stealing feed.

## 7.4 Young stock

Current rearing strategies for dairy cows aim at calving at the age of 24 months and a body weight between 550 and 575 kg after calving. Mourits et al. (1999) calculated that it was most profitable to aim at an average daily growth of 900 g/day and 700 g/day in pre-pubertal and pubertal animals, respectively. This strategy resulted in an age of 21.2 months at first calving and a body weight of 541 kg. These growth rates are much higher than the current recommendations of 800 g/day and 550 g/day in pre-pubertal (< 1 yr) and pubertal (>1 yr) animals, respectively. However, its practical significance has yet to be proven.

To achieve faster growth rates, energy intake should be improved which can be achieved by improved forage quality, a higher concentrate intake and more (by-pass) starch in the diet and the addition of fats and oils to the diet. This suggests that farmers can improve farm profitability while reducing at the same time the CH<sub>4</sub> emission from rumen fermentation in rearing heifers. A shorter rearing period will also cause a dilution of CH<sub>4</sub> per kg milk produced on the farm.

## 7.5 In conclusion

Increasing digestibility of the diet by means of more concentrates or better forage quality, addition of fats and oils and more (rumen) resistant starch is accompanied with higher energy density of the diet and an improved energy intake. Higher energy intake is beneficial for early lactation dairy cows (reduced NEB) and rearing heifers (faster growth rate). However, these dietary measures may cause overfeeding in low producing animals. Therefore, they should be applied with care and, for example, go together with a regular monitoring of the body condition score to avoid fat cows. It is also recommendable to keep cows in separate feeding groups according to the level of production (and nutritional needs). Separate feeding groups are also convenient to alternate dietary strategies in order to avoid adaptation. Feasibility of dietary measures to reduce CH<sub>4</sub> losses from dairy cattle with regard to different feeding groups are summarized in Table 3.

**Table 3** Feasibility of dietary measures to reduce CH<sub>4</sub> losses from dairy cattle with regard to different feeding groups

	<b>Early lactation</b>	<b>Mid-lactation</b>	<b>Late lactation / dry cows</b>	<b>Rearing cattle</b>
More concentrates	Low, inputs are already high	Moderate	Low, risk for overfeeding	Good, improving growth rates
Improved forage quality	Low, only little improvement possible	Moderate	Low, risk for overfeeding	Good, improving growth rates
More by-pass starch	Good, more mature maize silage, concentrate composition; helpful to reduce NEB	Moderate, risk for fat cows	Low, risk for overfeeding	Good, improving growth rates
Fats and oils	Good, helpful to improve milk quality, but need to avoid those additions that reduce milk quality	Good, helpful to improve milk quality, but need to avoid those additions that reduce milk quality	Low, risk for overfeeding	Good, improving growth rates
Secondary plant metabolites	Unknown	Unknown	Unknown	Unknown
Feed additives	Unknown	Unknown	Unknown	Unknown
Remarks		In mid lactation dairy cows, some restriction of feed intake is necessary to avoid too high body condition scores	The feasibility of these measures can be improved when the energy (feed) intake is restricted. This may require individual feeding systems	Improved growth rates have a risk of reduced milk yield in first lactation; requires careful balancing

## 8 General conclusions

Before results obtained in short term *in vitro* studies can be implemented in practice, an extensive evaluation with prolonged *in vivo* studies is needed to ascertain that no adaptation of the rumen microflora to the new situation occurs and nullifies the short term effects. Research on the sustainability *in vivo* of short term *in vitro* experiments should therefore receive a high priority.

Methane losses from dairy cows in The Netherlands are probably already among the lowest in the world and do most likely not exceed 6.0% of the GEI. An increase in milk yield per cow seems to be an almost autonomous development. As long as the milk quota system is maintained, methane losses per kg of milk will continue to diminish with an increased milk production per cow. This will, however, require high levels of concentrates and highly digestible diets balanced to meet the nutrient requirements of high yielding animals. It is expected that the content of starch, notably rumen resistant starch, in dairy diets will further increase. As a consequence, the reduction in CH<sub>4</sub> loss will be stimulated by the shift from rumen fermentation to intestinal digestion. The reduction in CH<sub>4</sub> loss, resulting from more concentrates with more starch may be offset by an increased use of fossil fuel in their production with the concomitant loss of CO<sub>2</sub>. Improving fertility and reducing the number of replacement animals can further reduce CH<sub>4</sub> losses. There is lack of quantitative knowledge on the degradative and passage behaviour of starch in the digestive tract of dairy cows, both in terms of site of digestion and in terms of VFA profile. This is considered a research area that deserves high priority, particularly starch in maize silage.

In specific situations the inclusion of lipids containing poly unsaturated fatty acids (PUFA) in dairy diets can reduce CH<sub>4</sub> losses, notably when fed in early lactation. At the same time it may improve the desired quality of the milk fat. However, supplements may reduce DMI and cell wall digestion. Long term and dose response production trials based on accurate measurements of CH<sub>4</sub> losses (preferably in climate respiration chambers), are needed to establish if the effects of the addition of fats on CH<sub>4</sub> losses in dairy diets are sustainable.

More research is also needed on the CH<sub>4</sub> depressing effects of forages, notably legumes, with a higher than normal content of condensed tannins. Research on sustainable *in vivo* effects of feed additives and secondary plant metabolites is still in its infancy.

Studies and strategies aiming at a reduction of the emission of GHG from dairy cows should be integrated and performed at farm rather than at animal level. Because current empirical models do not offer sufficient scope to evaluate methane mitigation options, they should be based on the use of dynamic mechanistic models and trade offs between different GHG should be taken into account. Current empirical models do not offer sufficient scope to evaluate methane mitigation options.

The fatty acid profile of milk seems an interesting and promising tool to monitor rumen fermentation, including CH<sub>4</sub> production.

Table 4 summarises the effects of the different interventions discussed in this report.

**Table 4** Summary of interventions aiming to reduce CH<sub>4</sub> losses in dairy cows

Intervention	Effect(s)	CH <sub>4</sub> loss	Additional effects	Practical feasibility
10% increase in milk yield	Dilution of maintenance	-5%	Higher concentrates, higher CO <sub>2</sub> loss	Moderate
8% less replacement animals	Less feed for maintenance	-2%	Less fresh grass, lower N <sub>2</sub> O loss	Very cost effective
10% Higher fertility,	Less feed for maintenance	-2%		Difficult
10% Better longevity	Less feed for maintenance	-2%		Difficult
Zero grazing	Better balanced diets	+3 %	Less fresh grass, lower N <sub>2</sub> O loss	High, but risks for welfare and consumer acceptance
Higher quality grass (silage)		0 %		Hardly feasible
Higher quality maize silage	Shift in rumen fermentation	*	More by-pass starch	High
Alternative (legume) forages		< -5%	Higher N <sub>2</sub> O loss	Low, legume swards are less persistent
More maize silage	Shift in rumen fermentation	*	Unlikely because of derogation	High for extensive farms, Moderate for derogation farms
More mature maize silage	Shift in site of digestion	< -5%	Easy and immediately applicable	High
Concentrates	Shift in rumen fermentation	< -5%	More concentrates, higher CO <sub>2</sub> loss	High, but risks for rumen acidosis, lameness, overfeeding
10% more				
with 10% more starch	Shift in rumen fermentation	< -5%	higher CO <sub>2</sub> loss ?	High, but risks for rumen acidosis
with 5% more resistant starch	Shift in site of digestion	< -5%	higher CO <sub>2</sub> loss?	High
with 10% less sugars	Shift in rumen fermentation	< -3%	higher CO <sub>2</sub> loss ?	High
home grown CCM,	Shift in rumen formation and site of digestion	*	Reduced CO <sub>2</sub> loss	High for extensive farms Low for intensive farms
Include fat in diet	Shift in rumen fermentation	< -5 %	Reduced cell wall digestion	Moderate, cost effectiveness ?
High oil maize	Shift in rumen fermentation	*	Increased NEL, milk production	Moderate but research needed
Tannins in (legume) forages	Reduce protozoa ?	?	Results in New Zealand positive	Low, but some potential for lotus
Plant secondary metabolites	Reduce protozoa ?	?	Only positive in vitro results	?
Essential oils	Reduce protozoa ?	?	Only (positive) in vitro results	?

\*: size of effect highly dependent on type of diet and feasibility highly dependent on farm management options

## Literature cited

- Argyle, J.L. & R.L. Baldwin, 1988. Modeling of rumen water kinetics and effects of rumen pH changes. *J. Dairy Sci.* 71, 1178-1188.
- Axelsson, J., 1949. The amount of produced methane energy in the European metabolic experiments with adult cattle. *Ann. R. Agric. Coll. Sweden.* 16, 404-419.
- Baldwin, R.L., Thornley, J.H.M. & D.E. Beever, 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *J. Dairy Res.* 54, 107-131.
- Baldwin, R.L., 1995. Modeling Ruminant Digestion and Metabolism. Chapman & Hall Ltd, London, UK.
- Bannink, A., De Visser, H & A.M. van Vuuren, 1997a. Comparison and evaluation of mechanistic rumen models. *Br. J. Nutr.* 78, 563-581.
- Bannink, A., De Visser, H., Klop, A., Dijkstra, J & J. France, 1997b. Impact of diet specific input parameters on simulated rumen function. *J. Theoret. Biol.*, 184, 371-384.
- Bannink, A., Dijkstra, J., Mills, J. A. N., Kebreab, E. & J. France, 2005a. Nutritional strategies to reduce enteric methane formation in dairy cows. In: Emissions from European Agriculture, pp. 367-376 [T. Kuczynski, U. Dämmgen, J. Webb and A. Myczko, editors]. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Bannink, A., Dijkstra, J., Mills, J.A.N, Kebreab, E. & J. France, 2005b. A dynamic approach for evaluating farm-specific as well as general policies to mitigate methane emissions by dairy cows. Proc. 4th Int. Symp. non-CO<sub>2</sub> Greenhouse Gases (NCGG-4). Science, Control, Policy & Implementation. A. Van Amstel, Coordinator. Millpress (www.millpress.nl), Utrecht, NL.
- Bannink, A. & S. Tamminga, 2005. Rumen Function. Pages 263-288 In: Quantitative Aspects of Ruminant Digestion and Metabolism, 2nd Edition. Eds. Dijkstra, J., J.M. Forbes & J. France. CAB International, Wallingford, UK.
- Bannink, A. & J. Dijkstra, 2005. Schatting van de vorming van vluchtige vetzuren uit gefermenteerd substraat in de pens van melkvee. ASG rapport 05/1002371, Lelystad, NL.
- Bannink, A. & J. Dijkstra, 2006. Voorspelling van de zuurgraad van pensvloeistof. ASG report 12. ASG, Lelystad
- Bannink, A., Kogut, J., Dijkstra, J., Kebreab, E., France, J., Tamminga, S. & A.M. Van Vuuren, 2006a. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *J. Theoret. Biol.*, 238, 36-51.
- Bannink, A., Dijkstra, J., Kebreab, E & J. France, 2006b. Advantages of a dynamical approach to rumen function to help resolve environmental issues. Pages 281-298. In: Modelling Nutrient Utilization in Farm Animals, Eds. E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits. CAB International, Wallingford, UK.
- Bannink, A., France, J., Lopez, S., Gerrits, W.J.J., Kebreab, E. Dijkstra, J & S. Tamminga, 2007. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. *Anim. Fd. Sci. Technol.* (in press).
- Bannink, A. & J. Dijkstra, 2007. Toevoeging van enkele onderdelen aan een dynamisch pensmodel [ weergave van voeropnamepatroon, vetzuurmetabolisme in de pens, en empirische vergelijkingen voor de voorspelling van darmvertering en melkproductie op basis van melksamenstelling ]. ASG rapport, Lelystad, NL. in prep.

- Barry, T.N., 1998. The feeding value of chicory (*Cichorium intybus*) for ruminant livestock. *J. Agric. Sci. Camb.*, 131, 251-257.
- Bauman, D.E., Mather, I.H., Wall, R.J. & A.L. Lock, 2006. Major advances associated with the biosynthesis of milk. *J. Dairy Sci.*, 89, 1235-1243.
- Beauchemin, K.A. & S.M. McGinn, 2005. Methane emissions from feedlot cattle fed barley or corn diets. *J. Anim. Sci.*, 83, 653-661.
- Beauchemin, K.A. & S.M. McGinn., 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oils, and canola oil. *J. Anim. Sci.*, 84, 1489-1496.
- Beever, D.E., 1993. Rumen function. Pages 187-215. In: Quantitative Aspects of Ruminant Digestion and Metabolism, Eds. J.M. Forbes & J. France. CAB International, Wallingford,
- Beever, D.E., Sutton, J.D., & C.K. Reynolds, 2001. Increasing the protein content of cow's milk. *Austr. J. Dairy Technol.*, 56, 138-149.
- Benchaar, C., Rivest, J., Pomar, C. & J. Chiquette, 1998. Prediction of methane production from dairy cows using existing mechanistic models and regression equations. *J. Anim. Sci.*, 76, 617-627.
- Bhatta, R., Krisnamoorthy, U. & F. Mohammed, 2000. Effect of feeding tamarind (*Tamarindus indica*) seed husk as a source of tannin on dry matter intake, digestibility of nutrients and production performance of crossbred dairy cows in mid-lactation. *Anim. Fd. Sci. Technol.*, 83, 67-74.
- Blaxter, K.L. & J.L. Clapperton, 1965. Prediction of the amount of methane produced by ruminants. *Br. J. Nutr.*, 19, 511-522.
- BLGG, 2006. Voederwaardes, [www.blgg.nl](http://www.blgg.nl)
- Boadi, D., Benchaar, C., Chiquette, J. & D. Massé, 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows. Update review. *Can. J. Anim. Sci.*, 84, 319-335.
- Bruinenberg, M.H., Van der Honing, Y., Agnew, R.E., Van Vuuren, A.M. & H. Valk, 2002. Energy metabolism of dairy cows fed on grass. *Livest. Prod. Sci.*, 75, 117-128.
- Cant, J.P., DePeters, E.J. & R.L. Baldwin, 1993. Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. *J. Dairy Sci.*, 76, 762-77.
- Carro, M.D. & M.J. Ranilla, 2003. Effect of the addition of malate on in vitro rumen fermentation of cereal grains. *Br. J. Nutr.*, 89, 181-188.
- Castillo, C., Benedito, J.L., Méndez, J., Pereira, V., López-Alonso, M., Miranda, M & J. Hernández, 2004. Organic acids as a substitute for monensin in diets for beef cattle. *Anim Fd. Sci. Technol.*, 115, 101-116.
- Chen, M. & M.J. Wolin, 1979. Effect of monensin & lasacoid-sodium on the growth of methanogenic & rumen saccharolytic bacteria. *Appl. Environ. Microbiol.* 38, 72-77.
- Chilliard, Y, Ferlay, A., Mansbridge, R.M. & M. Doreau, 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Ann. Zootech.*, 49, 181-205.
- Chilliard, Y., Ferlay, A., & M. Doreau, 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Review. *Livest. Prod. Sci.* 70, 31-48.

Corre, W. & G.J. Kasper, 2002. Beperking van lachgasemissie door gebruik van klaver in grasland. Alterra rapport 560.4, Alterra, Wageningen.

CVB, 2000. Chemical composition, digestibility and feeding value of feedstuffs (in Dutch). Centraal Veevoeder Bureau, Lelystad, The Netherlands.

Danfær, A., 2005. Methane emission from dairy cows. Pages 12-24 In: Evaluering af mulige tiltag til reduktion af landbrugets metanemissioner. Eds, J.E. Olesen, H. Jørgensen, A. Danfær, S. Gyldenkærne, M.H. Mikkelsen, W.H.M. Asman & S.O. Petersen. Arbejdsrapport fra Miljøstyrelsen 11 2005. Danmarks JordbrugsForskning. Miljøministeriet, Danmark.

Danfær, A., Huhtanen, P., Udén, P., Sveinbjornsson, J. & H. Volden, 2006. The Nordic Dairy Cow Model, Karoline – Description. Pages 383-406. In: Kebreab, Dijkstra, France, Bannink & Gerrits (eds). Modelling Nutrient Utilization in Farm Animals. CAB International, Wallingford, UK.

De Graeve, K. & D.I. Demeyer, 1988. Rumen and hindgut fermentation: differences for possible explanation? Med. Fac. Landbouww. Rijksuniv. Gent

Demeyer, D. & V. Fievez. 2000. Ruminants et environnement: la méthanogenèse. *Ann. Zootech* 49, 95-112.

DePeters, E.J. & J.P. Cant, 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. *J. Dairy Sci.*, 75, 2043-2070.

Dewhurst, R.J., Shingfield, K.J., Lee, M.R.F. & N.D. Scollan, 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Fd. Sci. Technol.*, 131, 168-206.

Dijkstra, J., Neal, H.D.St.C., Beever, D.E., & J. France, 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *J. Nutr.* 122, 2239-2256.

Dijkstra, J., 1994. Production and absorption of volatile fatty acids in the rumen. *Livest. Prod. Sci.*, 39, 61-69.

Dijkstra, J., France, J. & D.R. Davies, 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. *J. Dairy Sci.*, 81, 3370-3384.

Dijkstra, J. & A. Bannink, 2000. Analyses of modelling whole rumen function. Pages 299-322. In: Feeding Systems and Feed Evaluation Models, Eds. M.K. Theodorou & J. France, CAB International, Wallingford, UK.

Dijkstra, J., Gerrits, W.J.J., Bannink, A. & J. France, 2000. Modelling lipid metabolism in the rumen. Pages 25-236. In: Modelling Nutrient Utilization in Farm Animals. Eds. J.P. McNamara, J. France & D.E. Beever. CAB International, Wallingford, UK.

Dijkstra, J., Mills, J.A.N. & J. France, 2002. The role of dynamic modelling in understanding the microbial contribution to rumen function. *Nutr. Res. Rev.* 15, 67-90.

Dijkstra, J., Kebreab, E., Bannink, A., France, J. & S. Lopez, 2005. Application of the gas production technique in feed evaluation systems for ruminants. *Anim. Fd. Sci. Technol.*, 123-124, 561-578.

Dijkstra, J., Bannink, A., Van der Hoek, K.W. & W. Smink, 2006. Simulation of variation in methane emission in dairy cattle in The Netherlands. *J. Dairy Sci.*, 89, Suppl.1, 259.

Dijkstra, J., Kebreab, E., France, J. & A. Bannink, 2007. Modelling protozoal metabolism and VFA production in the rumen. Centre of Nutrition Modelling, University of Guelph, Canada, in press.



- Dohme, F., Machmüller, A., Wasserfallen, A & M. Kreuzer, 2000. Comparative efficiency of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with RUSITEC. *Can. J. Anim. Sci.*, *80*, 473-482.
- Dohme, F., Machmüller, A., Wasserfallen, A & M. Kreuzer, 2001, Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Letters in Appl. Microbiol.*, *32*, 47-51.
- Dohme, F., Machmüller, A., Sutter, F. & M. Kreuzer, 2004. Digestive and metabolic utilization of lauric, myristic and stearic acid in cows, and associated effects on milk fat quality. *Arch. Anim.Nutr.*, *58*, 99-116.
- Elgersma, A., Tamminga, S. & G. Ellen, 2006. Modifying milk composition through forage. *Anim. Fd. Sci. Technol.*, *131*, 207-225.
- Erasmus, L.J., Robinson, P.H., Ahmadib, A., Hinders, R. & J.E. Garrett, 2005. Influence of prepartum and postpartum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. *Anim. Fd. Sci. Technol.*, *122*, 219-239.
- Ferris, C.P., Gordon, F.J., Patterson, D.C., Porter, M.G. & T. Yan, 1999. The effect of genetic merit and concentrate proportion in the diet on nutrient utilization by lactating dairy cows. *J. Agric. Sci. (Camb)*, *132*, 483-490.
- Francis, G., Kerem, Z., Makkar, H.S.P. & K. Becker, 2002. The biological action of saponins in animal systems: a review. *Br. J. Nutr.*, *88*, 687-605.
- Friggens, N.C., Oldham, J.D., Dewhurst, R.J. & G. Horgan, 1998. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. *J. Dairy Sci.*, *81*, 1331-1344.
- Garnsworthy, P.C., 2004. The environmental impact of fertility in dairy cows: a modelling approach to predict methane and ammonia emissions. *Anim. Fd. Sci. Technol.*, *112*, 211-223.
- Giger-Reverdin, S., Morand-Fehr, P & G. Tran, 2003. Literature survey of the influence of dietary fat composition on methane production in dairy cattle. *Livest. Prod. Sci.*, *82*, 73-79.
- Givens, D.I. & K.J. Shingfield, 2004. Foods derived from animals: the impact of animal nutrition on their nutritive value and ability to sustain long-term health. *Nutr. Bulletin* *29*, 325-332.
- Hageman, I.W. & F. Mandersloot, 1994. Model energie verbruik melkveebedrijf. Publikatie nr. 86. Research Station for Cattle, Sheep and Horse Husbandry, Lelystad, The Netherlands.
- Harfoot, C.G. & G.P. Hazlewood, 1997. Lipid metabolism in the rumen. Pages 382-426 In: *The Rumen Microbial Ecosystem*, Second Edition, Eds: P.N. Hobson & C.S. Stewart. Blackie Academic & Professional, London.
- Hegarty, R.S., 2005. Genotype differences and their impact on digestive tract function of ruminants: a review. *Austr. J. Exp. Agric.*, *44*, 459-467.
- Hindrichsen, I.K., Wettstein, H.R., Machmüller, A. & M. Kreuzer, 2004. Enteric methane emission from dairy cows fed various diets and the corresponding methane emission from slurry. Pages 280-281 In: *Proc. Of the Internat. Conf. Greenhouse Gas Emission from Agriculture – Mitigation Options and Strategies* Ed. A. Weiske. Institute for Energy and Environment, Leipzig.
- Hindrichsen, I.K., Wettstein, H.R., Machmüller, A., Jørg, B. & M. Kreuzer, 2005. Effect of the carbohydrate composition of feed concentrates on methane emission from dairy cows and their slurry, *Enviren. Monit. Assess.*, *107*, 329-350.
- Holter, J.B. & A.J. Young, 1992. Methane prediction in dry and lactating Holstein cows. *J. Dairy Sci.*, *75*, 2165-2175.

Hristov, A.N., Ivan, M., Neill, L. & T.A. McAllister, 2003. Evaluation of several potential bioactive agents for reducing protozoal activity in vitro. *Anim. Fd. Sci. Technol.*, 105, 163-184.

Imamidoost, R. & J.P. Cant, 2005. Non-steady-state modeling of effects of timing and level of concentrate supplementation on ruminal pH and forage intake in high-producing grazing ewes. *J. Anim. Sci.*, 83, 1102-1115.

IPCC, 1996. Revised 1996 Guidelines for National Greenhouse Gas Inventories: Reference Manual. Chapter 4, Agriculture.

Ipharraguerre, I.R. & J.H. Clark, 2003. Usefulness of ionophores for lactating dairy cows: A review. *Anim. Fd. Sci. Technol.*, 106, 39-57.

Jenkins, T.C. & M.A. McGuire, 2006. Major advances in nutrition: impact on milk composition. *J. Dairy Sci.*, 89, 1302-1310.

Johnson, K.A. & D.E. Johnson, 1995. Methane emissions from cattle. *J. Anim. Sci.*, 73, 2483-2492.

Johnson, D.E. & G.M. Ward, 1996. Estimates of animal methane emission. *Environ. Monit. Assess* 42, 133-141.

Johnson, D.E., Ward, G.M. & G. Bernal, 1997. Biotechnology mitigating the environmental effects of dairying: Greenhouse gas emissions. Page 497-511 In: Milk composition, production and biotechnology, Eds. R.A.S. Welch, D.J.W. Burns, S.R. Davis, A.I. Popay, C.G. Prosser, CAB International, Wallingford, UK

Johnson, K.A., Kincaid, R.L., Westberg, H.H., Gaskins, C.T., Lamb, B.K. & J.D. Cronrath, 2002. The effect of oilseeds in diets of lactating cows on milk production and methane emissions. *J. Dairy Sci.*, 85, 1509-1515.

Jordan, E., Lovett, D.K., Monahan, F.J., Callan, J., Flynn, B. & F.P. O'Mara, 2006. Effect of refined coconut oil or copra meal on methane output and performance of beef heifers. *J. Anim. Sci.*, 84, 162-170.

Kodokawa, H., & B. Martin, 2006. A new perspective on management of reproduction in dairy cows: the need for detailed metabolic information and improved selection index and extended lactation. *J. Reprod. Developm.* 52, 161-168.

Kebreab, E., Mills, J.A.N., Crompton, L.A., Bannink, A., Dijkstra, J, Gerrits, W.J.J. & J. France, 2004. An integrated mathematical model to evaluate nutrient partition in dairy cattle between the animal and its environment. *Anim. Fd. Sci. Technol.*, 112, 131-154.

Kebreab, E., France, J., McBride, B.W., Bannink, A., Mills J.A.N. & J. Dijkstra, 2006a. Evaluation of models to predict methane emissions from enteric fermentation in North American Cattle. In: Modelling Nutrient Utilization in Farm Animals, Eds. E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits, CAB International, Wallingford, UK.

Kebreab, E., Clark, K., Wagner-Riddle & J. France, 2006b. Methane and nitrous oxide emissions from Canadian agriculture. A review. *Can. J. Anim. Sci.*, 86, 135-158.

Kirchgeßner, M., Windisch, W. & H.L. Muller, 1995. Nutritional factors for the quantification of methane production. Pages 333-348 In: Ruminant Physiology: Digestion, Metabolism Growth and Reproduction. Proc. 8<sup>th</sup> Int. Symp. on Ruminant Physiology Eds. W. von Engelhardt, S. Leonhard-Marek, GF. Breves & D Gieseke. Ferdinand Enke Verlag, Stuttgart.

Kirchgeßner, M., Windisch, W. & H.L. Muller. 1994. Methane release from dairy cows and pigs. Pages 399-402 In Proc. 13<sup>th</sup> Symposium on Energy Metabolism of farm Animals, Ed. J.F. Aguilera, EAAP publ. No. 76 CSIS Publ. Service, Spain.

- Klein Goldewijk, K., Olivier, J.G.J., Peters, J.A.H.W., Coenen, P.W.H.G. & H.H.J. Vreuls, 2005. Greenhouse gas emissions in the Netherlands 1990-2003. National Inventory Report 2005. RIVM Report 773201009/2005.
- Kohn, R.A. & R.C. Boston, 2000. The role of thermodynamics in controlling rumen metabolism. Pages 11-24 In *Modelling Nutrient Utilization in Farm Animals*. Eds. J.P. McNamara, J. France & D.E. Beever. CAB International, Wallingford, UK.
- Kolver, E.S., Aspin, P.W., Jarvis, G.N., Elborough, K.M. & J.R. Roche, 2004. Fumarate reduces methane production from pasture fermented in continuous culture. *Proc. NZ. Soc. Anim. Prod.*, *64*, 155-159.
- Kuikman, P.J., Oudendag, D., Smit, A & K.W. Van der Hoek, 2005. Mitigation of non-CO<sub>2</sub> greenhouse gas emission from agriculture: basis of design for effective communication and accounting of emissions. Proc. 4th Int. Symp. non-CO<sub>2</sub> Greenhouse Gases (NCGG-4). Science, Control, Policy & Implementation. A. Van Amstel, Coordinator. Millpress (www.millpress.nl), Utrecht, NL.
- LaCount, D.W., Drackley, D.J.K. Cicela, T.M. & J.H. Clark, 1995. High oil corn as silage or grain for dairy cows during an entire lactation. *J. Dairy Sci.*, *78*, 1745-1754
- Lescoat, P. & D. Sauvant, 1995. Development of a mechanistic model for rumen digestion validated using duodenal flux of amino acids. *Reprod. Nutr. Developm.*, *35*, 45-70.
- Lock, A.L. & D.E. Bauman, 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* *39*, 1197-1206.
- Maas, P.-J. 1987. Testing the calculation of the nutritive value according to the NEL system of freash grass with a high N content [In Dutch: Het testen van de voederwaardeberekening volgens het VEM-systeem met weidegras met hoge stikstofgehaltes.] MSc thesis Wageningen Agricultural University. 24 p.
- Manson F.J., & J.D. Leaver, 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. *Anim. Prod.*, *47*, 185-190.
- McGinn, S.M., Beauchemin, K.A., Coates, T., & D. Colombatto, 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast and fumaric acid, *J. Anim. Sci.*, *82*, 3346-3356.
- Mills, J.A.N., Dijkstra, J., Bannink, A., Cammell, S.B., Kebreab, E. & J. France, 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application. *J. Anim. Sci.*, *79*, 1584-1597.
- Mills, J.A.N., Kebreab, E., Yates, C.M., Crompton, L.A., Cammell, S.B., Dhanoa, M.S., Agnew, R.E. & J. France, 2003. Alternative approaches to predicting methane emissions from dairy cows. *J. Anim. Sci.*, *81*, 3141-3150.
- Moate, P.J., Clarke, T., Davis, L.H. & R.H. Laby, 1997. Rumen gases and bloat in grazing dairy cows. *J. Agric. Sci.*, *129*, 459-469.
- Moate, P.J., Chalupoa, W., Jenkins, T.C. & R.C. Boston, 2004. A model to describe ruminal metabolism and intestinal absorption of long chain fatty acids. *Anim. Feed Sci. Technol.*, *112*, 79-105.
- Moe, P.W. & H.F. Tyrrell, 1979. Methane production in dairy cows. *J. Dairy Sci.*, *62*, 1583-1586.
- Moss, A.R., Jouany, J.-P. & J. Newbold, 2000. Methane production by ruminants: its contribution to global warming. *Ann. Zootech.*, *49*, 2004, 231-253.
- Mourits, M.C.M., Huirne, R.B.M., Dijkhuizen, A.A. & D.T. Galligan, 1999. Optimal heifer management decisions and the influence of price and production variables. *Livest. Prod. Sci.*, *60*, 45-58.

- Murphy, M.R., Baldwin, R.L. & L.J. Koong, 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *J. Anim. Sci.*, *55*, 411-421.
- Murray, P.J., Gill, E., Baldson, S.L. & S.C. Jarvis, 2001. A comparison of methane emissions from sheep grazing pastures with differing management intensities. *Nutr. Cycl. Agroecosyst.*, *60*, 93-97.
- Newbold, C.J., McIntosh, F.M., Williams, P., Riccardo Losa & R.J. Wallace, 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. *Anim. Fd. Sci. Technol.*, *114*, 105-112.
- Newbold, C.J., Lopez, S., Nelson, N., Ouda, J.O., Wallace, R.J. and Moss, A.R., 2005. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation in vitro. *Brit. J. Nutr.* *94*, 27-35.
- Oba, M. & M.S. Allen, 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *J. Dairy Sci.*, *86*, 195-207.
- Offner, A. & D. Sauvant. 2006. Thermodynamic modelling of ruminal fermentations. *Anim. Res.* *55*, 1-23.
- Oudendag, D.N.A. & P.J. Kuikman, 2004. Handleiding ROB-DS versie 2.0; Decision support in het reductieplan Overige Broeikasgassen. Rapport 649 Alterra, Wageningen.
- Peaker, M., 1977. The aqueous phase of milk: ion and water transport. Pages 113-134, In: Comparative Aspects of Lactation, Ed. M. Peaker, London, Academic Press.
- Pen, B., Sar, C., Mwenya, B., Kuwaki, K., Morikawa, R. & J. Takahashi, 2006. Effects of *Yucca schidigera* and *Quillaja saponaria* extracts on *in vitro* ruminal fermentation and methane emission. *Anim Fd. Sci. Technol.*, *129*, 175-186.
- Pitt, R.E., Van Kessel, J.S., Fox, D.G., Pell, A.N., Barry, M.C. & P.J. Van Soest, 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. *J. Anim. Sci.*, *74*, 226-244.
- Puchala, R., Min, B.R., Goetsch, A.L. & T. Sahlu, 2005. The effect of a condensed tannin-containing forage on methane emission by goats. *J. Anim. Sci.*, *83*, 182-186.
- Ramirez-Restrepo, C.A. & T.N. Barry, 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. *Anim. Fd. Sci. Technol.*, *120*, 179-201.
- Reijs, J.W., A. Bannink, P. Bosma, E.A. Lantinga & J. Dijkstra. 2007. Modelling the effect of nutritional strategies for dairy cows on the composition of excreta N. Submitted.
- Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J. & C.J. Sniffen, 1992. A net carbohydrate and protein system for evaluating cattle diets: 1. Ruminal fermentation. *J. Anim. Sci.* *70*, 3551-3561.
- Saggar, S., Andrew, R.M., Tate, K.R., Hedley, C.B., Rodda, N.J. & J.A. Townsend, 2004. Modelling nitrous oxide emissions from dairy-grazed pastures. *Nutr. Cycl. Agroecosyst.* *68*, 243-255.
- Schils, R.L.M., Verhagen, A., Aarts, H.F.M. & L.B.J. Šebek, 2005. A farm level approach to define successful mitigation strategies for GHG emissions from ruminant livestock systems. *Nutr. Cycl. Agroecosyst.*, *71*, 163-175.
- Schils, R.L.M., Oudendag, D.A., Van der Hoek, K.W., De Boer, J.A., Evers, A.G. & M.H. De Haan, 2006a. Broeikasgasmodule BBPR. Animal Sciences Group, Praktijkrapport Rundvee 90.

- Schils, R.L.M., Verhagen, A., Aarts, H.F.M., Kuikman, P.J. & L.B.J. Sebek, 2006b. Effect of improved nitrogen management on greenhouse gas emissions from intensive dairy systems in the Netherlands. *Global Change Biology* 12: 382-391.
- Smink, W., Bos, K.D., Fitie, A.F., Van der Kolk, L.J., Rijm, W.K.J., Roelofs, G. & G.A.M. Van den Broek, 2003. Methaanreductie melkvee. Een onderzoeksrapport naar inschatting van de methaanproductie vanuit de voeding en naar de reductiemogelijkheden via de voeding van melkkoeien. FIS report, Wageningen.
- Smink, W., Pellikaan, W.F., Van der Kolk, L.J. & K.W. Van der Hoek, 2004. Methane production as a result from rumen fermentation in cattle calculated by using the IPCC-GPG Tier 2 method. FIS report, FS 04 12 E.
- Smink, W., Van der Hoek, K.W., Bannink, A & J. Dijkstra, 2005. Calculation of methane production from enteric fermentation in dairy cows. FIS-report, Wageningen.
- Sniffen, C.J., Ballarda, C.S., Cartera, M.P., Cotancha, K.W., Danna, H.M., Granta, R.J., Mandebvua, P., Suekawab, M. & S.A. Martin, 2006. Effects of malic acid on microbial efficiency and metabolism in continuous culture of rumen contents and on performance of mid-lactation dairy cows. *Anim. Fd. Sci. Technol.*, 127, 13-31.
- Staples, C.R., & W.W. Thatcher, 2001. Nutrient influences on reproduction of dairy cows. Proceedings of Mid-south Ruminant Nutrition Conference, pp. 21–35 (<http://www.txanc.org/proceedings.html#2001>).
- Sveinbjörnsson, J. 2006. Substrate levels, carbohydrate degradation rate and their effects on ruminal end-product formation. PhD Thesis. Acta Universitatis Agriculturae Sueciae 2006: 26. SLU, Uppsala, Sweden.
- Tamminga, S., Van Straalen, W.M., Subnel, A.P.J., Meijer, R.G.M., Steg, A., Wever, C.J.G. & M.C. Blok, 1994. The Dutch protein evaluation system: The DVE/OEB system. *Livest. Prod. Sci.*, 40, 139-155.
- Tamminga, S., 1996. A review on environmental impacts of nutritional strategies in ruminants. *J. Anim. Sci.* 74, 3112-3124.
- Tamminga, S., Aarts, F., Bannink, A., Oenema, O. & G.-J. Monteny, 2004. Actualiseren van geschatte N en P excreties voor rundvee. Reeks Milieu en Landelijk Gebied nr. 25, 1-48.
- Taweel, H.Z., Tas, B.M., Smit, H.J., Elgersma, A., Dijkstra, J. & S. Tamminga, 2005. Improving the quality of perennial ryegrass (*Lolium perenne* L.) for dairy cows by selecting for fast clearing and/or degradable neutral detergent fibre. *Livest. Prod. Sci.*, 96, 239-248.
- Thomassen, M.A., Van Calker, K.J., Smits, M.C.J., Iepema, G.L. & I.J.M. de Boer, 2007. Life Cycle Assessment of conventional and organic milk production in the Netherlands. *Agric. Ecosyst. Environ.*, in press
- Thornley, J.H.M. and J. France, 2007. Mathematical Models in Agriculture, 2nd edn. CABI Publishing, Wallingford, UK.
- Ulyatt, M.J. & K.R. Lassey, 2001. Methane emissions from pastoral systems: the situation in New Zealand. *Arch. Latinoam. Prod. Anim.*, 9, 118-126.
- Van Dijk, W., Groten, J., Van den Berg, W., Kassies, J. & J. Weijers, 2006. Efficiënt gebruik van snijmaïs Deel 2: invloed rastype en oogststadium op opbrengst en kwaliteit. Animal Sciences Group Wageningen-UR, Praktijk Rapport 85, pp 51.
- Van der Hoek, K.W. & M.W. Van Schijndel, 2006. Methane and nitrous oxide emissions from animal manure management, 1990 – 2003. Background document on the calculation method for the Dutch National Inventory Report. RIVM report 680125002; MNP report 500080002

- Van Knegsel, A.T.M., Van den Brand, H., Dijkstra, J., Tamminga, S. & B. Kemp, 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. A review. *Reprod. Nutr. Dev.* **45**, 665-688.
- Van Knegsel, A.T.M., Van den Brand, H., Dijkstra, J., Van Straalen, W.M., Heetkamp, M.J.W., Tamminga, S. & B. Kemp, 2007. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. *J. Dairy Sci.*, **90**, 1467-1476.
- Van Laar, H. & W.M. van Straalen, 2004. Ontwikkeling van een rantsoen voor melkvee dat de methaanproductie reduceert. Schothorst Feed Research
- Van Straalen, W.M., 2005. Voorspelling van de methaanproductie op een aantal praktijkbedrijven op basis van de rantsoensamenstelling en productieniveau. Schothorst Feed Research 740.
- Van Zijderveld, S. & W.M. van Straalen, 2004. Validatie van de IPCC methaan conversiefactor voor omstandigheden waaronder Nederlands melkvee wordt gehouden. Schothorst Feed Research proefverslag BET. 2004-29. [www.robklimaat.nl](http://www.robklimaat.nl)
- Veen, W.A.G., 2000. Veevoedermaatregelen ter vermindering van methaanproductie door herkauwers. Schothorst Feed Research proefverslag. [www.robklimaat.nl](http://www.robklimaat.nl)
- Verloop, J., Boumans, L.J.M., Van Keulen, H., Oenema, J., Hilhorst, G.J., Aarts, H.F.M., & L.B.J. Sebek, 2006. Reducing nitrate leaching to groundwater in an intensive dairy farming system. *Nutr. Cycl. Agroecosyst.* **74**, 59-74.
- Vlaeminck, B., Fievez, V., Cabrita, A.R.J., Fonseca, A.J.M. & R.J. Dewhurst, 2006. Factors affecting odd- and branched-chain fatty acids in milk, A review. *Anim. Fd. Sci. Technol.*, **131**, 389-417.
- Wallace, R.J., 2004. Antimicrobial properties of plant secondary metabolites. *Proc. Nutr. Soc.*, **63**, 621-629.
- Weiss, W.P. & D.J. Wyatt, 2000. Effect of oil content and kernel processing of corn silage on digestibility and milk production by dairy cows. *J. Dairy Sci.*, **83**, 351-358
- Whitlock, L.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F. & A.A. AbuGhazaleh, 2003. Milk production and composition from cows fed high oil or conventional corn at two forage concentrations. *J. Dairy Sci.*, **86**, 2428-2437
- Woodward, S.L., Laboyrie, P.J. & E.B.L. Jansen, 2000. Lotus corniculatus and condensed tannins - Effects on milk production by dairy cows. *Asian-Austral. J. Anim. Sci.*, **13**, 521-525.
- Woodward, S.L., Waghorn, G.C., & N.A. Thomson, 2006. Supplementing dairy cows with oils to improve performance and reduce methane- does it work ? *Proc. NZ Soc. Anim. Prod.* **66**, 176-181.
- Yan, T., Agnew, R.E., Gordon, F.J. & M.G. Porter, 2000. Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. *Livest. Prod. Sci.*, **64**, 253-263.
- Zom, R.L.G. , 2006. Efficiënt gebruik van snijmaïs Deel 5: invloed afrijpingstype en oogststadium op opname en melkproductie. Animal Sciences Group Wageningen-UR, Praktijk Rapport 88, pp 43.